The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

VOLUME 13 JANUARY, 1953 NUMBER 1
Copyright 1953 by The Endocrine Society

ON CERTAIN PHYSIOLOGIC RESPONSES TO INTRAVENOUS INJECTION OF CALCIUM SALTS INTO NORMAL, HYPERPARATHYROID AND HYPOPARATHYROID PERSONS*†

JOHN EAGER HOWARD, M.D., THEDA R. HOPKINS, M.D. AND THOMAS B. CONNOR, M.D.

From the Department of Medicine, The Johns Hopkins University and Hospital, Baltimore, Maryland

THE exact functional activities of the parathyroid hormone have not as yet been clearly defined, and it is often difficult for the physician to discern the functional status of the parathyroid glands. Some observations to be reported here on the response of normal individuals and of patients with abnormal parathyroid function to intravenous administration of calcium salts appear to throw some light on parathyroid physiology.

Previous studies have shown that calcium, inorganic phosphate and proteins in serum have certain specific relationships to one another, as was disclosed when additions of calcium and/or phosphate were made to pooled normal human serum, and the serum was then subjected to ultrafiltration (1). It was found that with a normal serum phosphorus level the concentration of calcium of the ultrafiltrate is approximately 70 per cent as high as that of the serum, and the phosphorus is almost wholly ultrafilterable until a serum calcium of 20 mg. per cent is reached. When the serum calcium level is raised above 20 mg. per cent, the ultrafilterabil-

Received for publication July 14, 1952.

* Read in abstract at the Annual Meeting of the Endocrine Society, Chicago, Illinois, June 5–7, 1952.

† The studies described in this paper were carried out under a contract between the Johns Hopkins University and the Atomic Energy Commission.

ity of serum phosphorus sharply diminishes and the calcium of the ultrafiltrate may or may not fall likewise. To check whether or not these same relationships hold *in vivo*, normal individuals were made hypercalcemic by intravenous administration of calcium salts. It was found that such hypercalcemic sera behaved during ultrafiltration in a manner identical to that of serum to which calcium had been added *in vitro*. However, the consistent finding of a rise in the concentration of serum phosphorus[1] and a fall in the urinary excretion of phosphorus[1] which occurred during and subsequent to the injection of calcium salts, arrested attention and prompted a study of the response of patients with parathyroid abnormalities to induced hypercalcemia.

PROCEDURE AND METHODS

The current pattern of the test was evolved gradually, as more experience was acquired. Earlier tests had been carried out with considerable variation in the routine. A constant diet was not employed; sometimes the infusion was administered with breakfast omitted; dosage of intravenous calcium varied from 11 to 19 mg. per kilo. None of these variations seemed to influence the results of the tests; yet a standard procedure adopted later was as follows: The individual under study ate an identical diet on two successive days, the total urine being collected for each day from 9:30 A.M. to 9:30 A.M. On the second day, approximately one hour after breakfast, there began an intravenous infusion. This flow was so regulated that the liter of saline solution containing calcium as a salt (usually gluconate-glucoheptonate[2]), 15 mg./Kg., was administered at a steady rate for a four-hour period, and the patient was allowed to eat lunch at the usual time during the infusion. Blood for serum calcium, phosphorus and total serum protein determinations was obtained immediately before infusion, in the middle and at the end of the infusion, four hours after the infusion ended and twenty-four hours after the start of the infusion. The study was carried out on the Metabolism Ward; efforts were made to have the patients' activity the same on the two days. Dietary management was under the direction of Miss Helen Lincoln, who personally supervised the meals. Drinking water was allowed without restriction. When, as occasionally happened, the induced hypercalcemia caused some anorexia or nausea at the luncheon meal, the moiety not eaten was fed in mid-afternoon or with the evening meal. Chemical methods used were as follows: serum calcium (2), serum phosphorus (3), serum chloride (4), serum CO_2 (5),

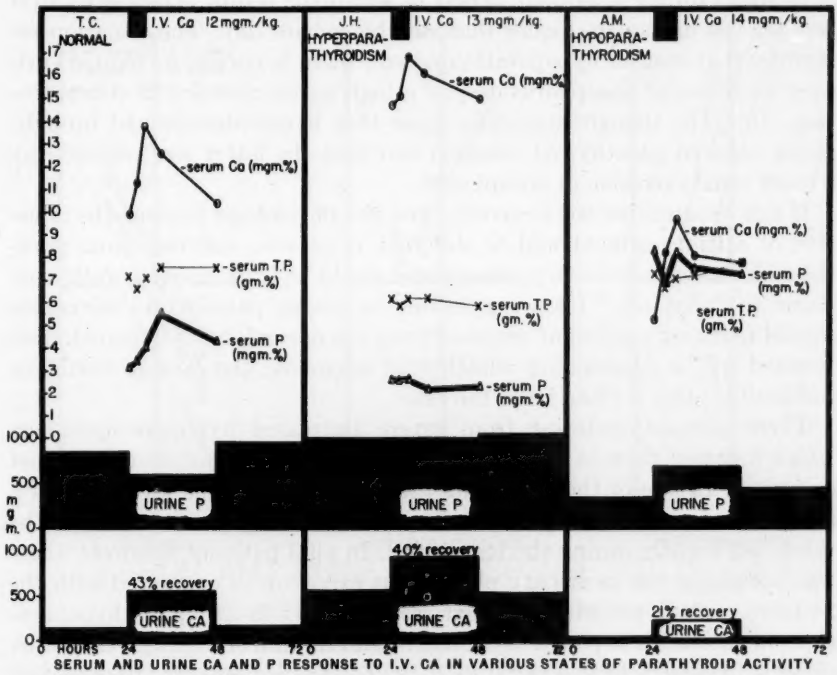
[1] The terms serum and urinary phosphorus are used throughout to denote the inorganic phosphorus of the serum and urine.

[2] Calcium gluconate-glucoheptonate solution (equivalent in calcium content to calcium gluconate 10 per cent W/V) was supplied through the courtesy of Dr. Arnold E. Osterberg, Abbott Laboratories, Research Division, North Chicago, Illinois.

total protein (6), urinary calcium (7), urinary phosphorus (3), hematocrit (8), urinary sodium and potassium (9). Ultrafiltration was carried out as in the previous report from this laboratory (1).

RESULTS

In 8 "normal" persons tested, the results ran in strikingly parallel fashion. At the end of the four-hour injection period, the serum calcium level had been raised by 1.3 to 5.8 mg. per 100 cc.; four hours later it



SERUM AND URINE CA AND P RESPONSE TO I.V. CA IN VARIOUS STATES OF PARATHYROID ACTIVITY

FIGURE 1

had fallen somewhat, and by the next morning had returned to the control figure or only slightly above.

The serum phosphorus level invariably rose during the calcium infusion; the maximum observed rise occurred at the end of the infusion in half of the patients, four hours later in the remainder. The serum phosphorus had fallen in all instances by the following morning but in 6 instances was still higher at this time than the control value. At the end of the period of fluid administration, there was always apparent a slight hemodilution, manifested by small reductions of the hematocrit and in concentration of serum proteins.

The 24-hour urinary excretion of phosphorus was invariably *less* on the experimental day than on the control day (Fig. 1, left-hand column). Urinary volume was nearly always *greater* on the experimental day than on the control day. Twenty-four-hour collections of urine were used for the comparison of phosphorus excretion between control and experimental days, because four, six and eight-hour specimens did not reflect the difference in phosphorus excretion with such great consistency.

The reduction in urinary phosphorus excretion was most striking, since it occurred during a 24-hour period in which the serum phosphorus level was for the most part higher than on the control day. The situation resembles that seen in hypoparathyroidism; there is normal or reduced urinary excretion of phosphorus despite a high serum phosphorus concentration (10). The thought naturally arose that hypercalcemia had brought about reduced parathyroid function and that the latter was reflected by a lesser renal excretion of phosphorus.

If this explanation were correct, then the physiologic response to injection of calcium salts should be different in persons suffering from parathyroid hormone deficiency, since there would be little if any parathyroid tissue to "shut off." Likewise persons harboring parathyroid adenomas should manifest a different response from the normal, for if hypercalcemia "turned off" a functioning parathyroid adenoma, the disease would be self-limited; this is clearly not the case.

Three persons, suffering from severe untreated hypoparathyroidism which followed thyroid surgery, were given calcium intravenously as just described. In one of these patients the serum phosphorus level showed a maximum rise of 1.9 mg. per cent; in another, 0.8 mg. per cent; and in the third, fell slightly during the test period. In all 3 patients, however, there was a striking *rise* in urinary phosphorus excretion as compared with the 24-hour control period. A typical response in this group of hypoparathyroid patients is depicted in the right-hand column of Figure 1. One other surgical hypoparathyroid patient (M.K.) had to be discharged unexpectedly, so that only the urine secreted during infusion was obtained. Fortunately on the control day her urine had been partitioned. Comparison between the two four-hour periods showed a rise in urinary phosphorus of 100 per cent on the test day. Two patients with so-called "idiopathic" hypoparathyroidism were tested and their responses will be discussed later.

Five patients, subsequently proven to have parathyroid adenomas, were subjected to similar intravenous calcium tests. Among these, the serum phosphorus level rose in 3, but the maximum rise was 1 mg. per cent. The serum phosphorus fell 0.3 and 0.4 mg. per cent in the other 2 patients.

In the patients with parathyroid adenomas, the 24-hour urinary phosphorus, as compared with that of the control day, rose in 1, remained the

same in 2, and fell 14 and 6 per cent in the 2 others. The middle column of Figure 1 shows a characteristic response in this group.

The differences in the behavior of serum and urinary phosphorus between the normal subjects and those with parathyroid dysfunctions are brought out more clearly in Figure 2. In these graphs the serum phosphorus rise (or fall) in mg. per cent at eight hours after beginning the test is plotted to the left of the midline. Equidistant to the right of the midline

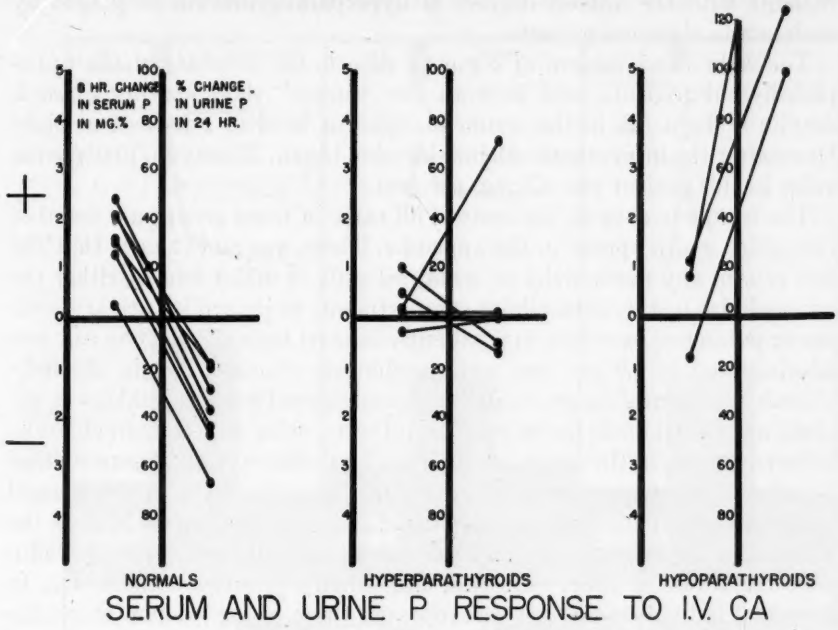

8 HR. CHANGE IN SERUM P IN MG.%
% CHANGE IN URINE P IN 24 HR.
NORMALS
HYPERPARATHYROIDS
HYPOPARATHYROIDS
SERUM AND URINE P RESPONSE TO I.V. CA

FIG. 2. (For method of charting, *see* text.)

is plotted the rise (or fall) of urinary phosphorus in percentile variation from the control day. The parallelism of the lines drawn between these points for the "normal" persons (left column, Fig. 2) is remarkable. The consistency of the response is dependent upon the fact that those persons who showed the highest rise in the serum phosphorus level had the least percentile fall in urinary phosphorus. One would expect that a rise in a readily filterable substance would result in increased glomerular filtration of that substance and a greater urinary output if other factors remained the same.[3] Increased tubular reabsorption of phosphorus must have occurred to a degree relatively proportional to the quantitative increase in

[3] This actually occurred when phosphorus was administered intravenously, as will be shown later.

glomerular filtrate phosphorus, in order that the "curves" in these "normal" patients should have run so nearly parallel. But the important thing is that all individuals in this group showed *reduced* urinary phosphorus on the test day, and the "curves" all slope sharply downward.

In the hyperparathyroid group (Fig. 2, middle column), no one of the curves slopes downward as markedly as does that of any normal; only two curves slope downward at all, and these represent the responses of the patients with the mildest degrees of hyperparathyroidism as judged by their serum chemical patterns.

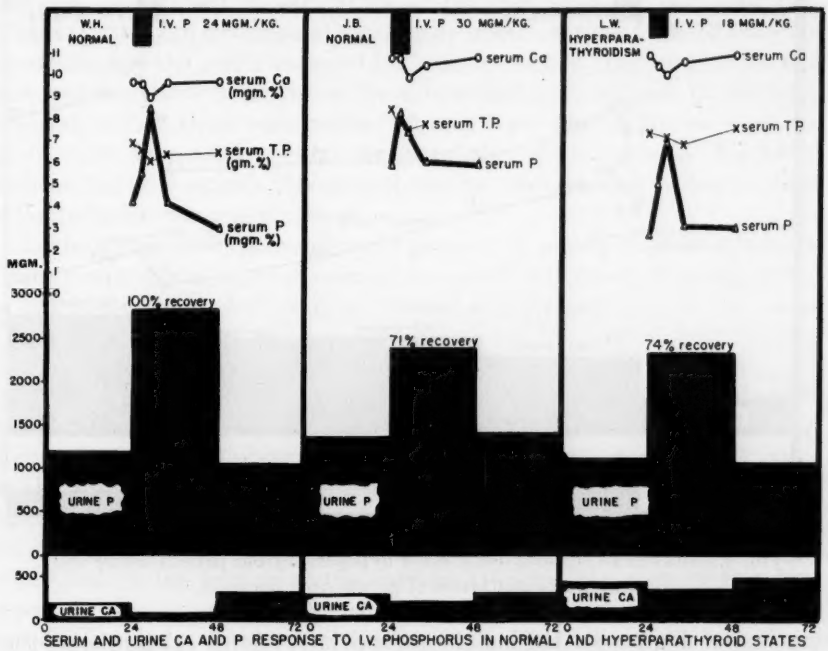
The right hand column of Figure 2 depicts the response of the hypoparathyroid patients, and here all the "curves" slant sharply *upward*, despite a slight *fall* in the serum phosphorus level in 1 patient at eight hours after the intravenous calcium infusion began. The serum phosphorus value in this patient was 8.3 mg. per cent.

The complete data on the tests of all three of these groups are listed in the tables which appear in the appendix. There was no evidence that the test caused any noteworthy or sustained shift in major ions of either the extracellular or the intracellular compartment, as judged by urinary sodium or potassium excretion in 6 patients. In most tests the calcium salt was administered in 0.9 per cent sodium chloride solution; and in the individuals on whom data are available the additional sodium intake was entirely accounted for in the urine. Control tests, using only sodium chloride, induced no rise in the serum phosphorus level and no conspicuous change in urinary phosphorus excretion. Some of the earlier tests in the normal subjects were run, using 5 per cent glucose instead of sodium chloride as the solvent for the calcium salt; and this substitution did not appear to influence the resulting effect on serum and urinary phosphorus behavior. In 1 normal individual the test was repeated twice, using calcium gluconate in 0.9 per cent saline and calcium chloride in normal saline. The behavior of serum calcium and phosphorus and urinary phosphorus during the two tests was similar to that observed in the other tests carried out on normal persons. On this same person the test was performed, using sodium bicarbonate in 0.9 per cent sodium chloride, and administering the same number of milliequivalents of sodium as of calcium in a previous test. There was no comparable rise in the serum phosphorus level and no significant change in urinary phosphorus excretion (Patient T.C., Table 1 of Appendix).

Isotonic sodium phosphate (1,650 mg. of phosphorus), pH 7.35, was administered over a four-hour period to 2 normal subjects and to 1 patient with a parathyroid adenoma (Fig. 3) In each instance the serum phosphorus level was elevated by approximately 4 mg. per cent above the control level, and there was brisk phosphorus diuresis, so that nearly all the injected phosphorus could be accounted for in the 24-hour urine of the test

day. The total serum calcium concentration fell slightly during administration of the fluids, *but no more than could be accounted for by hemodilution,* as judged by proportional reduction in hematocrit and serum protein concentration. Urinary calcium, however, fell in all 3 patients on the test days, as compared with the control days.

An effort was made to determine the effect of exogenous parathyroid hormone on the response to the intravenous calcium test. A patient (Fig. 4) with chronic hypoparathyroidism was studied a) while receiving no



SERUM AND URINE CA AND P RESPONSE TO I.V. PHOSPHORUS IN NORMAL AND HYPERPARATHYROID STATES

FIGURE 3

therapy, and b) during intramuscular administration of large doses of parathyroid hormone (Parathormone, Lilly—300 I.U. every six hours). The patient was on a constant diet throughout the five days of the experiment. During the first test, the serum calcium level rose from 5.3 to 10 mg. per cent and the serum phosphorus level rose from 4.9 to 6.8 mg. per cent. Urinary excretion of phosphorus doubled on the test day, as compared with the control, and 14 per cent of the injected calcium was recovered in the urine during the twenty-four hours of the test day. Parathyroid hormone was then given for forty-eight hours prior to the second test and continued during the twenty-four hours of the second test period. It will be noted that the effect of parathyroid hormone was much more apparent

on serum and urinary phosphorus than on calcium. The serum phosphorus level fell steadily from 4.9 to 1.3 mg. per cent while urinary phosphorus remained elevated (having risen as the result of the first intravenous calcium test). The serum calcium level, however, was only 1.5 mg. per cent higher than on the control day after seven doses of Parathormone at six-hour intervals, and urinary calcium excretion remained minimal (*see* bot-

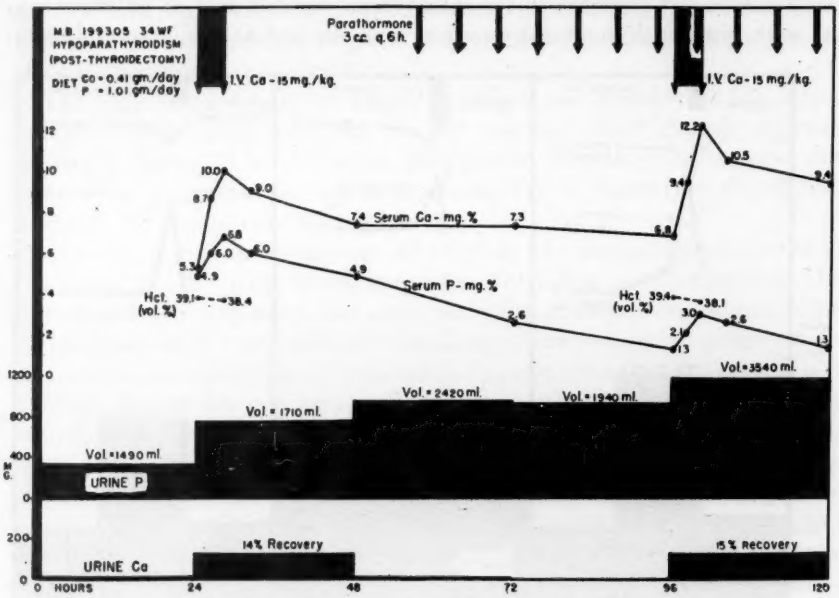


FIG. 4. Intravenous calcium test given a hypoparathyroid patient before and during administration of parathyroid hormone.

tom line of Figure 4). During the second intravenous calcium test, the serum phosphorus level rose from 1.3 to 3 mg. and urinary phosphorus rose still further above the baseline. It is interesting that the quantity of calcium recovered in the urine was identical on the two test days, indicating perhaps that the skeletal receptivity toward calcium had not been changed in this hypoparathyroid patient by this dosage of parathyroid hormone over a 48-hour period.

Urinary calcium

The quantity of calcium appearing in the urine was invariably greater during the twenty-four hours in which the calcium was administered intravenously. This quantitative increase has been calculated in the tables (*see* Appendix) as the percentage of the administered dose which appeared in the urine during the test day. In the "normal" individuals, from 36 to

53 per cent of the test dose of calcium appeared in the urine (as excess calciuria compared with the control day). In the hyperparathyroid patients the range of variation was from 75 per cent to 32 per cent, but the patient with 75 per cent urinary calcium recovery was the only one to excrete relatively more calcium in the urine on the test day than did the "normals." None of these hyperparathyroid patients had clinically demonstrable skeletal disease. However, when tested from four days to six weeks after removal of the parathyroid adenomas, the urinary calcium recovered after the test dose varied widely—from 72 per cent to 6 per cent recovery. This variation did not depend apparently upon the time elapsing between operation and the performance of the test; for in L.W. the test was carried out four days after removal of the tumor and recovery was 72 per cent, whereas in G.S. there was recovery of only 9 per cent of the injected calcium eight days after spontaneous infarction of her tumor. This latter patient had mild osteitis fibrosa as shown by sternal biopsy at the time the infarcted adenoma was removed.

In the 3 surgical hypoparathyroid patients on whom data are available, urinary recovery of injected calcium was uniformly lower than that found in any of the "normals,"—only 21, 14 and 13 per cent.

DISCUSSION

In 1924 Salvesen (11) noted that the serum phosphorus level rose when calcium salts were administered intravenously to the dog, and in 1950 Bassett's group (12) observed the same phenomenon in man. The latter workers suggested that the elevation of the serum phosphorus level might be explained by a cessation of parathyroid function induced by the hypercalcemia, since on other evidence it appeared that parathyroid function is controlled by the level of serum calcium (13–15). The observations here reported, that urinary phosphorus excretion falls, despite the rise of the serum phosphorus level, when calcium salts are administered to normal individuals, serves to strengthen still further the hypothesis that normal parathyroid functional activity is governed by the calcium concentration of the extracellular fluid.

It seems clearly established that the parathyroid hormone influences renal reabsorption of inorganic phosphate. This is demonstrated most easily in patients with severe degrees of parathyroid insufficiency, in whom urinary phosphorus excretion may increase 50-fold in response to an intravenous dose of parathyroid hormone (16). When there is already a normal quantity of parathyroid hormone present, a further phosphorus diuretic effect of parathyroid hormone may be difficult to demonstrate (16–19).

With parathyroidectomy, urinary phosphorus excretion rapidly diminishes (20a) despite a rising concentration of serum phosphorus, and the urinary phenomenon observed in our "normal" patients seems to resemble

closely that which follows parathyroidectomy. The pattern of observed response in "normals" runs a strikingly stereotyped course in all individuals so far given the intravenous calcium test. The similarity of response is further accentuated by the fact that the charted curves of serum and urine phosphorus changes are amazingly parallel, even though in earlier experiments the "test" was varied, in that breakfast was fed to some and not to others, the calcium was administered in 5 per cent glucose instead of normal saline, and some of the patients were ambulatory[4] and not on constant diets.

The fall in urinary phosphorus excretion during the test day in the normal group cannot be explained on the basis of reduced ultrafiltrability of the serum phosphorus. Before, and at the height of serum calcium concentration (*i.e.*, at end of injection) ultrafiltration was performed on sera obtained from several patients (1). Though small reductions in ultrafilterable phosphorus were observed, similar reductions were manifest in sera from hyper- and hypoparathyroid patients during the tests; yet in these latter groups urinary phosphorus excretion either rose, or fell less, than did that of the normal group (Table 4).

Patients with later proven parathyroid adenomas responded differently from the normal subjects, in that the average rises in the levels of serum calcium and phosphorus were less and the urinary excretion of phosphorus was smaller, unchanged or even greater, as compared with that of the control day. Of the hyperparathyroid patients, those with the mildest degrees of the malady, as judged by serum chemical abnormalities, manifested "curves" which were closest to those of the normal group. One would not anticipate that a parathyroid tumor would be as easily "shut off" as would normal parathyroid tissue, by elevation of the calcium of its environment. It is believed that the function of normal parathyroid glands is suppressed by a functioning adenoma; in weakly functioning tumors the normal glands may be still further suppressed by the hypercalcemia resulting from the test; or the tumor itself may be partly under control of the calcium concentration in its environment.

Interpretation of the urinary phosphorus response in the hypoparathyroid patients is more difficult. In three surgical hypoparathyroid individuals, urinary phosphorus excretion was greatly augmented on the test day, though in one of these no rise in the concentration of serum phosphorus was detected. Two patients with *idiopathic* hypoparathyroidism (both sensitive to parathyroid hormone) were given intravenous calcium tests. One of these, an adult, who was receiving large doses of oral calcium chloride during the control and test days in order to avoid active tetany, responded with a rise in both serum and urinary phosphorus. The other

[4] These patients came to the hospital only for the four-hour period of intravenous injection; otherwise they pursued their normal activities.

patient was a 10-year-old child, who likewise was receiving oral calcium supplement; during the test there was a slight fall in the serum phosphorus level and also a smaller urinary phosphorus excretion than on the control day. When plotted as in Figure 2, this patient's response was almost within the normal range. This child had an unexplained elevation of serum alkaline phosphatase (27 Bodansky units), and recalls the patient reported by Albright (20b), who manifested a fall in serum and urinary phosphorus when given calcium intravenously over a six-day period. It is suspected that both these hypoparathyroid patients, Albright's and our own, may have hypocalcemic rickets.

When one examines the data on serum phosphorus elevation resulting from the intravenous administration of calcium in normal subjects, it becomes apparent that the rise in the serum phosphorus level cannot be accounted for by the reduced urinary output of phosphorus. Since the phosphorus in serum has been found to be nearly completely ultrafilterable (1) and hence freely diffusible and rapidly equilibrated (21) within the extracellular compartment, an increment of approximately 240 mg. of phosphorus must be provided to account for a rise in serum phosphorus concentration of 2 mg. per cent[5] in an individual of average size.

The maximum rise in the level of serum phosphorus was often found at the end of the injection period, and 240 mg. of phosphorus could not have been saved by renal excretion in this period, even if no phosphorus had been excreted. Though in our present studies the 24-hour urinary collections were not partitioned in most instances, the urine excreted during the injection period was examined in several cases; in some the excretion of phosphorus was found to be reduced, but in others it was actually greater than the average four-hourly excretion of the control day. It was for this reason that, in evaluating results of the test, the 24-hour urinary phosphorus has been used for comparison with that of the control day.

The source of the phosphorus which brings about the rise in serum concentration is, therefore, of considerable interest and can only be speculated upon at this time. It seems unlikely that ingested phosphorus lying in the gut is the source of the increment, because the same rise in the serum phosphorus concentration followed intravenous calcium administration when the test was performed in the fasting state (eighteen hours postprandial), as when the patient was given breakfast. It seems unlikely also that the skeleton, a rich storehouse of phosphorus, would yield phosphate in response to hypercalcemia, since hypercalcemia would tend toward deposition rather than release of lime salt. The total turnover and exchange rate

[5] If one were to postulate that the inorganic phosphorus of the cellular compartment rose similarly, there would then have to be 600 mg. of additional inorganic phosphorus provided from somewhere, in order for the serum inorganic phosphorus to rise 2 mg. per cent.

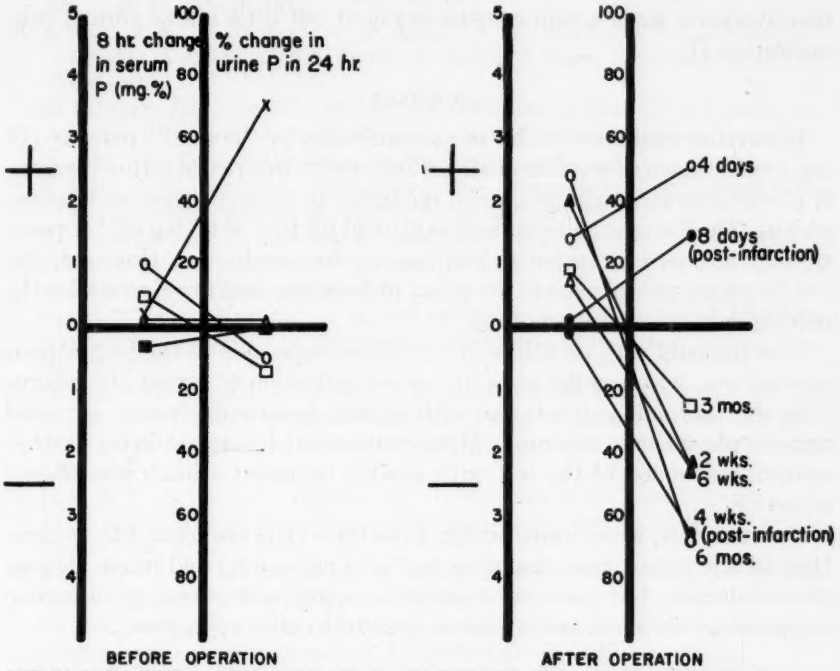
of phosphate in all the body's metabolic processes is known to be large, and a small change in either cellular utilization of phosphate or acceleration in discharge rate of inorganic phosphorus from the cellular compartment could easily account for a considerable rise in the level of serum phosphorus. One is left thus with the most reasonable explanation that elevation of the serum calcium level per se causes a shift of cellular phosphate to the extracellular compartment. Such a shift would most likely result from an effect on a phosphate enzyme system.

The 5 patients with parathyroid adenomas were tested several weeks or months after removal of the tumors. One of these patients (R.C.) at this time continued to have a slightly elevated serum calcium level (11.5 mg.), a low serum phosphorus (2.5 mg.) and hypercalciuria. Her response to the test still deviated from the normal (Fig. 5—line labelled "3 mos."), and another parathyroid adenoma was sought and removed just prior to this writing.[6] The other 4 patients, normocalcemic and apparently euparathyroid, gave responses in serum and urinary phosphorus to the test (Fig. 5) which were similar to those of the normal group (for details, *see* tables in Appendix) when plotted according to the method used in Figure 2. It is of some interest that one of these patients, L.W., six months after operation, still had a serum phosphorus level just as low as it had been before removal of the tumor, a situation which may exist for months (22). Despite this low initial concentration of serum phosphorus, this patient responded to intravenous calcium administration with a rise of 2.4 mg. per cent in the serum phosphorus value and a 68 per cent fall in urinary phosphorus excretion, as compared with his control day. The serum alkaline phosphatase content was only slightly elevated, and there was never in this patient any radiographic evidence of skeletal disease, nor was there manifest osteitis fibrosa in the sternal biopsy specimen which was taken at operation.

Also in Figure 5 are shown tests made in 2 individuals very early after their hyperparathyroidism had been corrected. Both these patients showed abnormal responses. In the former instance (L.W.) a parathyroid adenoma had been removed four days previously; and, at the time of the test, the serum calcium level had fallen from 11.2 to 9.6 mg. per cent, and the serum phosphorus level had risen from 2.7 to 4.1 mg. per cent. Six months later this same patient gave a normal response to the test. The other patient had been admitted to the hospital seriously intoxicated by hyperparathyroidism and there were present also symptoms and signs of duodenal ulcer. The concentrations of serum calcium and phosphorus were 12.7 and 3.5 mg. per cent respectively, that of the nonprotein nitrogen was slightly elevated (67 mg. per cent) and there was mild alkalosis from vomit-

[6] Two months after the second adenoma was removed, this patient responded to an intravenous calcium test, with a normal pattern.

ing. Under observation and during rehydration the serum calcium level rose to 20.2 mg. per cent and then fell precipitously over the course of three days to 10.7 mg. per cent. A diagnosis was made of parathyroid adenoma with probable infarction, and this was subsequently verified at operation, when there was removed a tumor (3.5×2×2 cm.), completely necrotic save for a thin zone of normal-appearing parathyroid cells around the



SERUM AND URINE P RESPONSE TO I.V. CA BEFORE AND AFTER OPERATION FOR REMOVAL OF PARATHYROID ADENOMA

FIG. 5. (Method of charting, as in Figure 2—*see* text.)

periphery. Unfortunately, no test was performed prior to infarction, but eight days after the precipitous fall in the serum calcium content, when serum calcium and phosphorus levels were 8.2 and 1.8 mg. per cent respectively, the test was carried out and showed a sharp rise in urinary phosphorus excretion as compared with the control day. When the test was repeated fourteen days after operation (twenty-seven days after infarction), the concentrations of serum calcium and phosphorus were 9.3 and 1.4 mg. per cent, respectively; and though the serum phosphorus level rose only 0.3 mg. per cent as a result of the infusion, there was a sharp

fall in urinary phosphorus excretion, giving a "normal" response curve.

It is hoped that others will repeat and amplify the experimental observations here reported. Caution is suggested in administering calcium salts to persons who already have marked hypercalcemia, or hyperphosphatemia without coincident hypocalcemia, since marked changes in ultrafiltrability of calcium and phosphorus occur when the concentration of serum calcium exceeds 20 mg. per cent with normophosphatemia, and also with hyperphosphatemia when serum calcium is raised but little above normal concentration (1).

SUMMARY

Intravenous administration of calcium salts to "normal" persons (15 mg. per kilogram over a four-hour period) results in a rise in serum inorganic phosphorus concentration and a reduction in urinary excretion of phosphorus. The latter is believed best explained by the "shutting off" of parathyroid hormone secretion in response to hypercalcemia. However, the rise in serum phosphorus is too great to be accounted for entirely by the reduction in urinary phosphorus.

Five patients with parathyroid adenomas responded to the intravenous calcium test with smaller rises in the concentration of serum phosphorus than the "normal" subjects and with either a lesser reduction or increased urinary phosphorus excretion. Three patients with surgical hypoparathyroidism responded to the test with greatly increased urinary phosphorus excretion.

Two patients, when tested within a few days after removal of their parathyroid adenomas, gave responses in the hyperparathyroid range, despite normocalcemia; but these same patients, as well as 3 others, gave normal responses when tested two weeks to six months after operation.

REFERENCES

1. Hopkins, T.; Howard, J. E., and Eisenberg, H.: Ultrafiltration studies on calcium and phosphorus of human serum, *Bull. Johns Hopkins Hosp.* (to be published).
2. Clark, E. P., and Collip, J. B.: A study of the Tisdall method for the determination of blood serum calcium with a suggested modification, *J. Biol. Chem.* **63**: 461, 1925.
3. Umbreit, W. W.; Burris, R. H., and Stauffer, J. F.: Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Minneapolis, Minn., Burgess Publishing Co., 1945, p. 103.
4. Schales, O., and Schales, S. S.: A simple and accurate method for the determination of chloride in biological fluids, *J. Biol. Chem.* **140**: 879, 1941.
5. a) Van Slyke, D. D., and Cullen, G. E.: The bicarbonate concentration of the blood plasma, its significance and its determination as a measure of acidosis, *J. Biol. Chem.* **30**: 289, 1917.
   b) Van Slyke, D. D., and Stadie, W. C.: The determination of the gases of the blood, *J. Biol. Chem.* **49**: 1, 1921.

6. Campbell, W. R., and Hanna, M. I.: The determination of nitrogen by modified Kjeldahl methods, *J. Biol. Chem.* **119:** 1, 1937.
7. Consolazio, C. F.; Johnson, R. E., and Marek, E.: Metabolic Methods; Clinical Procedures in the Study of Metabolic Function. St. Louis, C. V. Mosby Co., 1951, p. 95.
8. Wintrobe, M. M.: Macroscopic examination of the blood; discussion of its value and description of the use of a single instrument for the determination of sedimentation rate, volume of packed red cells, leucocytes and platelets and of icteric index, *Am. J. M. Sc.* **185:** 58, 1933.
9. a) Barnes, R. B.; Richardson, D.; Berry, J. W., and Hood, R. L.: Flame photometry. A rapid analytical procedure, *Indust. & Engin. Chem.* (analyt. ed.) **17:** 605, 1945.
   b) Howard, J. E., and Bigham, R. S., Jr.: Potassium determination by flame photometer. Tr. 11th Conf. on Metabolic Aspects of Convalescence, Oct. 15–16, 1945. New York, Josiah Macy, Jr., Foundation, 1946, p. 9.
   c) Hald, P. M.: The flame photometer for the measurement of sodium and potassium in biological materials, *J. Biol. Chem.* **167:** 499, 1947.
10. Albright, F., and Ellsworth, R.: Studies on the physiology of the parathyroid glands. I. Calcium and phosphorus studies on a case of idiopathic hypoparathyroidism, *J. Clin. Investigation* **7:** 183, 1929.
11. Salvesen, H. A.; Hastings, A. B., and McIntosh, J. F.: The effect of the administration of calcium salts on the inorganic composition of the blood, *J. Biol. Chem.* **60:** 327, 1924.
12. Baylor, C. H.; Van Alstine, H. E.; Keutmann, E. H., and Bassett, S. H.: The fate of intravenously administered calcium. Effect on urinary calcium and phosphorus, fecal calcium and calcium-phosphorus balance, *J. Clin. Investigation* **29:** 1167, 1950.
13. Ham, A. W.; Littner, N.; Drake, T. G. H.; Robertson, E. C., and Tisdall, F. F.: Physiological hypertrophy of the parathyroids, its cause and its relation to rickets, *Am. J. Path.* **16:** 277, 1940.
14. Patt, H. M., and Luckhardt, A. B.: Relationship of a low blood calcium to parathyroid secretion, *Endocrinology* **31:** 384, 1942.
15. Stoerk, H. C., and Carnes, W. H.: The relation of the dietary Ca:P ratio to serum calcium and to parathyroid volume, *J. Nutrition* **29:** 43, 1945.
16. Ellsworth, R., and Howard, J. E.: Studies on the physiology of the parathyroid glands. VII. Some responses of normal human kidneys and blood to intravenous parathyroid extract, *Bull. Johns Hopkins Hosp.* **55:** 296, 1934.
17. Jahan, E., and Pitts, R.: Effect of parathyroid on renal tubular reabsorption of phosphate and calcium, *Am. J. Physiol.* **155:** 42, 1948.
18. Handler, P.; Cohn, D. V., and De Maria, W. J. A.: Effect of parathyroid extract on renal excretion of phosphate, *Am. J. Physiol.* **165:** 434, 1951.
19. Milne, M. D.: Observations on the action of the parathyroid hormone, *Clin. Sc.* **10:** 471, 1951.
20. a) Albright, F., and Reifenstein, E. C., Jr.: Parathyroid Glands and Metabolic Bone Disease. Baltimore, Md., The Williams & Wilkins Co., 1948.
   b) *Ibid.*, p. 126.
21. Kleiber, M.; Smith, A. H.; Ralston, N. P., and Jasper, D. E.: Mixing rate within the mobile phosphate pool of cows, *Fed. Proc.* **8:** 86, 1949.
22. a) Albright, F.: Personal communication.
   b) Unpublished data of the authors.

APPENDIX

TABLE 1. RESULTS OF ADMINISTRATION OF INTRAVENOUS CALCIUM TO NORMAL PERSONS

Subject	Age, Race, Sex	Diet	Status*	Dose of I.V. Ca		Initial conc. in serum			Rise in Ca, mg.%	Change in serum P		Urine P				Urine Ca				Urine volume			Remarks
				Total mg.	mg./ Kg.	Ca, mg.%	P, mg.%	T. Prot., Gm.%		4th hr., mg.%	8th hr., mg.%	Control day, mg.	Test day, mg.	Change, %	Post-test day, mg.	Control day, mg.	Test day, mg.	Recovery, %	Post-test day, mg.	Control day, ml.	Test day, ml.	Post-test day, ml.	
T.R.H.	28 W F	Constant; Ca=0.73 Gm., P=1.04 Gm.	F	960	19	10.0	3.7	6.6	5.8	+1.4	+2.1	735	465	−37	—	77	589	53	—	1,850	2,800	—	
V.S. 454179	19 W F	Not constant	N.F.	870	13	10.1	3.8	8.3	3.4	+1.4	+1.3	715	380	−47	855	241	552	36	266	1,375	2,390	750	I.V. Ca given in 1,330 cc. 5% glucose in H_2O
E.W. 153979	55 C F	Not constant	N.F.	455	11	9.1	3.4	7.4	1.3	+0.4	+0.3	85	29	−66	100	29	201	38	43	520	1,130	590	
C.F. 579168	44 C M	Not constant	F	970	16	9.9	4.6	6.7	3.5	+1.1	+1.5	1,098	885	−19	1,178	232	699	48	253	1,220	1,595	1,320	
B.P. 580648	37 W M	Not constant	F	1,080	13	10.1	4.4	7.3	3.5	+0.7	+1.6	873	490	−44	911	94	464	34	88	5,410	5,115	2,960	I.V. Ca given in 900 cc. 5% glucose in H_2O
J.S. 250708	18 C M	Constant; Ca=1.41 Gm., P=2.89 Gm.	N.F.	1,177	15	11.2	3.9	8.5	2.9	+2.2	+2.1	1,228	1,008	−18	1,310	151	680	45	174	1,915	2,900	1,780	
E.P. 607977	51 C M	Constant; Ca=0.29 Gm., P=0.85 Gm.	N.F.	874	16	10.2	4.5	7.5	2.1	+1.6	+1.4	740	426	−42	843	28	328	34	41	2,960	1,800	1,900	
T.B.C.	30 W M	Constant; Ca=1.31 Gm., P=1.82 Gm.	F	990	12	9.9	3.1	7.2	3.9	+0.9	+2.4	848	600	−29	—	137	559	43	—	1,200	2,060	—	I.V. Ca given as calcium gluconate
T.B.C.	30 W M	Constant; Ca=0.22 Gm., P=0.93 Gm.	N.F.	845	11	9.9	3.1	7.1	3.2	+1.5	+1.5	734	496	−32	891	70	485	49	114	825	1,570	1,310	I.V. Ca given as $CaCl_2$
T.B.C.	30 W M	Constant; Ca=0.22 Gm., P=0.93 Gm.	N.F	—	—	10.4	2.8	7.1	†	+0.6	+0.7	858	890	+ 4	734	64	87	—	70	1,395	1,615	825	58 cc. 7.5% $NaHCO_3$ given in 940 cc. saline i.v.

* F=Fasting for 15 hours prior to and during i.v. calcium infusion period.
N.F.=Test begun 30 to 60 minutes after breakfast; dinner given during infusion period.
† Serum calcium fell 1.1 mg.% in second hour; 0.5 mg.% in fourth hour.

TABLE 2. RESULTS OF ADMINISTRATION OF INTRAVENOUS CALCIUM TO PATIENTS WITH PARATHYROID ADENOMAS

Subject	Time tested	Age, Race, Sex	Diet	Status*	Dose of I.V. Ca		Initial conc. in serum			Rise in serum Ca, mg. %	Change in serum P		Urine P				Urine Ca				Urine volume			Remarks
					Total mg.	mg./Kg.	Ca, mg. %	P, mg. %	T. Prot., Gm. %		4th hr., mg. %	8th hr., mg. %	Control day, mg.	Test day, mg.	Change, %	Post-test day, mg.	Control day, mg.	Test day, mg.	Recovery, %	Post-test day, mg.	Control day, ml.	Test day, ml.	Post-test day, ml.	
B.B. 575980	Pre-op.	57 W F	Ca constant <0.2 Gm./day	F	600	9	13.0	2.6	6.5	2.3	+0.4	+0.2	444	760	+71	268	254	743	81	193	1,485	3,240	1,120	
L.W. 440159	Pre-op.	51 W M	"	F	920	10	11.2	2.7	7.8	2.6	+0.7	+1.0	1,080	1,015	− 6	1,108	430	755	35	440	3,680	4,755	3,310	
L.W. 440159	4 days post-op.	"	"	F	730	8	9.6	4.1	7.8	1.5	+0.8	+1.4	1,112	1,676	+51	2,170	183	709	72	478	1,365	3,170	3,280	
L.W. 440159	6 mos. post-op.	"	Constant; Ca=0.27 Gm., P=1.06 Gm.	N.F.	1,329	14	9.4	2.4	7.3	4.5	+1.5	+2.4	1,068	343	−68	—	196	614	32	—	3,580	5,270	—	Persistent urinary tract infection
J.H. 149997	Pre-op.	57 C M	Ca content, <0.2 Gm./day	F	990	13	14.7	2.6	6.2	2.1	0	−0.4	1,014	1,014	0	1,110	557	952	40	535	2,530	4,390	2,655	
J.H. 149997	6 wks. post-op.	"	"	F	865	11	9.4	3.8	6.9	1.9	+0.7	+0.7	839	463	−45	—	8	56	6	—	1,230	1,435	—	
G.S. 563600	8 days post-infarction	57 C F	Ca content, less than 0.5 Gm./day	F	950	20	8.2	1.6	7.1	4.0	+0.5	+0.1	79	102	+29	—	13	93	8	—	910	1,710	—	NPN=47 mg. %
G.S. 763600	27 days post-infarction (14 days post-op.)	"	"	F	880	19	9.3	1.4	7.1	3.6	+0.3	−0.1	16	6	−63	—	32	172	16	—	2,335	1,960	—	NPN=29 mg. %
H.B. 487048	*Cf.* remarks	52 C F	"	N.F.	531	15	14.6	2.5	7.4	1.8	+0.1	−0.3	502	503	0	—	274	437	31	—	400	655	—	NPN=50 mg. %. Tested 2 days before Death from metastatic melanosarcoma. Parathyroid adenoma found at autopsy. Bones not involved.
D.S. 557218	2 wks. post-op.	30 W F	Not constant	N.F.	452	9	9.6	3.5	7.3	2.4	+0.9	+2.0	449	251	−44	631	79	258	40	54	2,910	3,100	1,830	I.V. Ca given in 1,220 cc. 5% glucose in H_2O.
R.C. 515217	Pre-op.	23 W F	Ca constant, 0.29 Gm./day	F	830	13	12.1	2.4	7.4	2.5	0	+0.5	1,030	890	−14	750	561	848	35	518	1,630	2,290	1,170	Test performed before first adenoma was removed
R.C. 515217	3 mos. after 1 adenoma was removed, but before 2nd was excised	"	Constant; Ca=0.19 Gm., P=0.76 Gm.	F	886	14	11.5	2.5	7.1	2.7	+0.8	+0.9	890	664	−25	845	225	510	32	229	985	1,245	715	

* Same as in Table 1.

Table 3. Results of administration of intravenous calcium to patients with hypoparathyroidism

Patient	Diagnosis	Age, Race, Sex	Diet	Status*	Dose of I.V. Ca		Initial conc. in serum			Rise in serum Ca, mg. %	Change in serum P		Urine P				Urine Ca				Urine volume			Remarks
					mg.	mg./Kg.	Ca, mg. %	P, mg. %	T. Prot., Gm. %		4th hr., mg. %	8th hr., mg. %	Control day, mg.	Test day, mg.	Change, %	Post-test day, mg.	Control day, mg.	Test day, mg.	Recovery, %	Post-test day, mg.	Control day, ml.	Test day, ml.	Post-test day, ml.	
A.M. 256843	Post-surg. hypoparathyroidism	40 C F	Not constant	N.F.	1,010	14	5.3	8.3	7.3	4.4	−0.1	−0.8	346	687	+ 99	453	35	242	21	37	2,390	3,710	2,760	
M.B. 199305	"	37 W F	Constant Ca=0.45 Gm., P =1.07 Gm.	N.F.	827	15	5.3	4.9	7.1	4.7	+1.9	+1.1	340	762	+124	—	11	128	14	—	1,490	1,705	—	
M.B. 199305	"	"	"	N.F.	865	15	6.8	1.3	7.1	5.4	+1.7	+1.3	935	1,180	+ 26	—	4	133	15	—	1,940	3,540	—	After Rx—Parathormone, 3 cc. i.m. q. 6 hrs.×11 (*see* Fig. 5)
E.P. 602354	"	47 W F	Constant Ca=0.16 Gm., P=0.64 Gm.	N.F.	735	16	5.6	5.9	6.5	3.8	+0.5	+0.8	60	173	+189	109	60	154	13	15	595	1,155	1,025	
A.C. 229986	Idiopathic hypoparathyroidism	40 W F	Constant Ca=0.21 Gm., P=0.71 Gm.	N.F.	755	16	7.0	6.2	5.9	4.6	+0.7	−0.1	192	809	+320	—	253	760	67	—	1,950	3,160	—	$CaCl_2$, 18 Gm. q.d. by mouth
C.H. A91669	"	10 C F	Not constant	N.F.	582	16	6.3	8.3	8.1	3.5	−0.1	−0.4	151	63	− 58	13	7	26	3	3	1,230	1,530	330	Ca lactate, 16.8 Gm. q.d. by mouth
M.K. 429779	Post-surg. hypoparathyroidism	21 W F	Not constant	F.	825	12	5.7	6.9	7.0	5.8	+0.2	+0.3	55†	112†	+104	—	1*	28†	—	—	180†	804†	—	

* Same as in Table 1.

† Four-hour collections.

TABLE 4. ULTRAFILTRATION STUDIES ON THE SERUM OF PATIENTS GIVEN INTRAVENOUS CALCIUM

Patient No.	Diagnosis	Filtrate, pH	Serum Ca, mg. %	Filtrate Ca, mg. %	Per cent filtrate Ca	Serum P, mg. %	Filtrate P, mg. %	Per cent filtrate P	T. Prot. Gm. %	Time serum obtained
C.F. 579168	Normal	7.58	9.9	6.7	68	4.6	5.2	113	6.7	Pre-infusion.
		7.24	13.4	9.8	73	5.7	5.6	98	—	End of infusion.
		7.52	10.1	7.5	74	5.0	5.7	114	6.7	Twenty hrs. post-infusion.
B.P. 580648	Normal	7.42	10.1	7.0	69	4.4	5.0	114	7.3	Pre-infusion.
		7.36	13.6	10.4	76	5.1	5.4	106	6.7	End of infusion.
L.W. 440159	Parathyroid adenoma	7.53	11.2	7.4	66	2.7	3.1	115	7.8	Pre-infusion.
		7.58	13.8	8.4	61	3.4	3.4	100	7.1	End of infusion.
		7.52	11.6	7.5	65	3.0	3.6	120	7.6	Twenty hrs. post-infusion.
J.H. 149997	Parathyroid adenoma	7.68	15.8	10.7	68	2.7	3.1	115	6.5	Pre-infusion.
		7.56	16.8	12.6	75	2.6	2.0	77	6.2	End of infusion.
		7.53	15.0	10.7	71	2.3	2.7	117	5.9	Twenty hrs. post-infusion.
A.C. 229986	Idiopathic hypoparathyroidism	7.47	6.1	4.3	70	5.5	6.3	115	5.8	Pre-infusion.
		7.40	11.6	9.7	84	6.9	7.3	106	5.7	End of infusion.
M.K. 429779	Post-surgical hypoparathyroidism	7.52	5.7	3.7	65	6.9	7.5	109	7.0	Pre-infusion.
		7.52	11.5	5.0	43	7.1	6.0	85	7.0	End of infusion.

MULTIPLE ENDOCRINE ADENOMAS: REPORT OF 8 CASES IN WHICH THE PARATHYROIDS, PITUITARY AND PANCREATIC ISLETS WERE INVOLVED*

LAURENTIUS O. UNDERDAHL, M.D., LEWIS B. WOOLNER, M.D. AND B. MARDEN BLACK, M.D.

From the Divisions of Medicine, of Surgical Pathology, and of Surgery, Mayo Clinic, Rochester, Minnesota

THE occurrence of a pituitary tumor, islet-cell adenoma and parathyroid tumor or of any combination of two of these in the same patient is extremely rare. It is the primary purpose of this paper to present the total experience of the Mayo Clinic with cases of this syndrome. To date, 8 cases of multiple adenomas involving two or all three of the aforementioned endocrine organs have been studied; 5 were surgical and 3 were necropsy cases.

REVIEW OF LITERATURE

A review of the literature was carried out to determine the total number of cases in which tumors of the pituitary body, pancreatic islets and parathyroid glands, or of any two of these organs, occurred in the same patient. Data on these cases, 14 in number, are given in Table 1. This table includes cases in which any two or all three of these endocrine glands were affected and in which the tumors were clinically hyperfunctioning or were of sufficient size to be of clinical significance. Occasionally, in a case of primary hyperparathyroidism or hyperinsulinism with islet-cell tumor micro-adenomas, 1 to 2 mm. in diameter, were found in the pituitary at necropsy. Such cases have been omitted from the table but are described briefly in the text. Also excluded from the table were those cases in which an association of endocrine tumors was mentioned but no clinical or pathologic data were provided. The 14 cases listed in Table 1 will be discussed briefly.

Pituitary tumor with associated parathyroid adenomas.—The first mention of acromegaly with eosinophilic adenoma of the pituitary associated with four enlarged parathyroids was made by Erdheim in 1903 (1). No details of the necropsy findings are given and the parathyroids are not

Received for publication June 7, 1952.

* Read at the combined meeting of the American Diabetes Association and The Endocrine Society, Chicago, Illinois, June 7, 1952.

TABLE 1. MULTIPLE ENDOCRINE ADENOMAS INVOLVING THE PITUITARY, PARATHYROIDS AND PANCREATIC ISLETS: CASES REPORTED IN THE LITERATURE, 1903 TO 1951, EXCLUSIVE OF MAYO CLINIC CASES

Author, reference, year	Age, sex	Pituitary	Parathyroids	Pancreatic islets	Miscellaneous
Erdheim (1), 1903*		Acromegaly. Acidophilic adenoma	Enlarged. 12×5×3 mm. 8×8×3 mm. 17×6×3 mm. 11×6×4 mm.	Not described	
Claude & Baudouin (2), 1911*	22 F	Acromegaly. Eosinophilic adenoma, 48 Gm.	No clinical data. Four enlarged: two, size of kidney bean; two, size of small hazelnut	Not described	Adrenals, 17 Gm. Thyroid, 190 Gm. Hyperthyroidism clinically
Josefson (3), 1915*	26 M	Acromegaly. Large eosinophilic adenoma	No clinical data. Two adenomas: one, size of bean; one, 6×4×2.5 cm.	No data	Adrenals, 40 Gm. Adenomatous formations
Cushing & Davidoff (4), 1927*	35 M	Acromegaly. Huge eosinophilic adenoma	No clinical data. Two parathyroids found; central adenoma in each	Diabetes, which disappeared. Adenoma, 1 cm. Hypertrophied islets	Adrenals, combined wt., 30 Gm. Thyroid, 100 Gm.
Lloyd (5), 1929*†	22 F	Inoperable pituitary tumor. Necropsy: chromophobe adenoma, 3×2×1.5 cm.	No clinical data. Two enlarged glands: 1.7×1×0.8 cm., 1.5 ×0.4×0.3 cm.	No clinical data. One nodule, 10×7×6 mm.; eight nodules, 3 to 6 mm. in diameter	Adrenals "large and firm," 12 Gm. each
Hadfield & Rogers (6), 1932*	51 M	Acromegaly. Anterior lobe enlarged	No clinical data. Large mass behind one lateral lobe of thyroid, 8×4×2.5 cm. (64 Gm.)	Not described	
Kalbfleisch (7), 1937*†	43 M	X-ray: tumor. Necropsy: chromophobe adenoma	One chief-cell adenoma. Other three glands size of pea, microscopically hyperplastic	Hyperinsulinism. Plum-sized tumor removed at operation. Necropsy: four additional islet-cell adenomas; hyperplasia of islets	Nodule size of egg in each lobe of thyroid
Gerstel (8), 1938*	36 M	Acromegaly. Eosinophilic adenoma, size of walnut	No clinical data. Single lobular adenoma, chief-cell type, size of hen's egg	Islet-cell tumor in tail, size of fist. Multiple microscopic islet-cell adenomas	Perforated duodenal ulcer. Pinealoma. Nodular enlargement of both adrenal cortices. Nodular goiter
Shelburne & McLaughlin (9), 1945†	26 M	X-ray: intrasellar tumor. Defect of visual field	Hyperparathyroidism. Small adenoma removed	Hyperinsulinism. Three islet-cell adenomas removed surgically: diameters 1.5, 1, and 0.5 cm.	Duodenal ulcer
Rutishauser & Guirguis (10), 1945*	46 F	Acromegaly. Eosinophilic adenoma, 2.85 Gm.	Not described	No clinical data. Multiple small islet-cell adenomas, largest 6×5 mm.	
Burk (11), 1948†	47 F	Acromegaly	Hyperparathyroidism. Tumor removed at operation, size not stated	No clinical data	
Burk (11), 1948†	30 M		Hyperparathyroidism. Tumor removed at operation, size not stated	Hyperinsulinism. Tumor removed at operation, size not stated	
Crain & co-workers (12), 1949†	46 F	Acromegaly. X-ray: enlarged sella turcica	No clinical or chemical data	Hyperinsulinism. Two islet-cell adenomas removed surgically: 8×3×1.3 cm., 1.1 ×1×0.6 cm.	
de Ulhôa Cintra & co-workers (13), 1952†	28 F	Chromophobe adenoma removed at operation	Hyperparathyroidism. Adenoma about 3×1 cm. removed at operation	No clinical evidence of hypoglycemia	

* Tumors encountered at necropsy.

† Tumors encountered at operation.

sufficiently described to enable one to classify the enlargement pathologically.

A similar case of acromegaly and associated parathyroid adenomas was described by Claude and Baudouin (2) and another was described by Josefson (3), in both of which multiple parathyroid adenomas were present. In the case of Hadfield and Rogers (6), a single large parathyroid mass was described. The other parathyroids were not mentioned.

The first reported case of acromegaly and clinical hyperparathyroidism with removal of a parathyroid tumor was that of Burk (11) in 1948. No description of the pathologic characteristics of the parathyroid tumor is provided nor are any details of the postoperative course given.

A case of chromophobe adenoma of the pituitary surgically removed, with associated hyperparathyroidism, was reported by de Ulhôa Cintra and co-workers (13). A single parathyroid adenoma was removed at operation. Two and a half years postoperatively there were still generalized osteoporosis, many renal calculi, persistent hypophosphatemia and hypercalcemia. The authors discuss the hypophosphatemia as being likely related to the pituitary lesion but do not mention the possibility of another parathyroid tumor. The latter is probable.

Acromegaly and islet-cell adenomas.—The association of acromegaly and islet-cell tumors has been described in 2 instances. One case of this type was encountered by Rutishauser and Guirguis (10). In their case an acromegalic with a previous history of "insulin-resistant diabetes" was found at necropsy to have multiple islet-cell adenomas (largest, 6 by 5 mm.) and associated hyperplasia of the islets of Langerhans. No further details are given and no mention is made of the parathyroids.

Crain and co-workers (12) reported the case of an acromegalic who had hypoglycemic episodes and who at operation was found to have two islet-cell adenomas. During the immediate postoperative period, the patient required insulin but later the concentration of fasting blood sugar was essentially normal.

Parathyroid tumor and islet-cell adenoma.—This association of hyperfunctioning endocrine tumors was reported by Burk (11). A single parathyroid tumor and a single islet-cell adenoma were removed at separate operations. No pathologic data or details of the postoperative course are given.

Associated tumors of the pituitary, parathyroid and pancreatic islets.—This remarkable association of endocrine tumors has been described only 5 times in the literature.

In 1927, Cushing and Davidoff (4) in a study of 4 acromegalics at necropsy observed a huge eosinophilic adenoma of the pituitary. Two associated small adenomas of the parathyroids were present. The pancreas

contained an adenoma described as "acinar" but which, from the accompanying photomicrograph, had the appearance of an islet-cell adenoma. The remaining islets of Langerhans were hypertrophied, measuring up to 400 microns in diameter.

In Lloyd's (5) case there was an inoperable pituitary tumor of chromophobe type, and at necropsy multiple adenomas were found both in the parathyroids and in the pancreatic islets. There were no clinical data relating to the parathyroids or pancreas and no blood chemical determinations were made.

In the case studied by Kalbfleisch (7) there was roentgenologic evidence of a pituitary tumor and clinical evidence of hypoglycemia. A plum-sized islet-cell tumor was removed surgically. At necropsy two days later, four additional islet-cell adenomas and multiple hyperplastic islets three or four times normal size were found. A pituitary tumor of chromophobe type extended out of the sella. In one parathyroid gland there was an adenoma of the chief-cell type. The remaining three glands were described as pea sized; microscopically, there was evidence of hyperplasia with loss of fatty stroma. There was a left renal calculus, the kidneys being otherwise normal. The bones were soft and could be cut with a knife.

Gerstel (8) reported on a patient with acromegaly who died after an operation for a perforated duodenal ulcer. At necropsy an acidophil adenoma of the pituitary was found. A single, lobular, chief-cell adenoma of the parathyroid was present. No other parathyroids were identified. The pancreas contained one large, and multiple microscopic islet-cell tumors.

The first reported case of associated pituitary tumor, hyperfunctioning parathyroid tumor and hyperfunctioning islet-cell tumors, all recognized clinically and treated, was that of Shelburne and McLaughlin (9) in 1945. The patient in this case had hypoglycemia clinically, for which exploration was carried out, with removal of three islet-cell adenomas; there was apparent cure. Renal calculi were removed surgically from both kidneys and the concentration of blood calcium was found to be 13.5 mg. per 100 cc. A single small parathyroid adenoma was removed, after which the value for serum calcium returned to normal. Roentgenograms revealed evidence of a pituitary tumor with a defect of the visual field on the right. Roentgen therapy was given.

Multiple endocrine adenomas of small size or inadequately documented.— In addition to the 14 cases listed in Table 1, 3 cases of primary hyperparathyroidism have been described in which associated micro-adenomas of the pituitary or pancreatic islets, or both, were present at necropsy. These include a case reported by Wernly and Berdjis-Chamsi(14) in 1946, in which a parathyroid adenoma, the size of a large nut, was surgically

removed. At necropsy micro-adenomas, one eosinophilic and one chromophobic, were found in the pituitary. In the pancreas a single islet-cell adenoma, measuring 3.5 by 2 mm., was found. The islets were increased in size and number. Although it is realized that rarely an adenoma of this size can produce hyperinsulinism, there apparently were no such symptoms in this case.

Black and Ackerman (15) in a review of 23 cases of hyperparathyroidism mentioned a case in which there were associated adenomas of unstated size, of the pancreas, adrenals and kidneys. In a second case there were incidental findings of a 1-mm. eosinophilic adenoma in the pituitary and two 2-mm. islet-cell adenomas in the pancreas.

Malamud and Grosh (16) reported a case of hyperinsulinism in which, at necropsy, an islet-cell tumor of the pancreas and a small adenoma of the pituitary were found. An essentially similar case was reported by Rienhoff and Lewis (17) and 2 similar cases were reported by Friedman (18).

Kretschmer (19) in reporting 4 cases of parathyroid adenomas associated with renal calculi, mentioned the necropsy finding of an adenocarcinoma of insular tissue in 1 case. No details were given.

Chasnoff and co-workers (20) reported a case of acromegaly in which there was clinical hyperparathyroidism but because of impaired renal function, no operation on the parathyroids was performed.

Crain and co-workers (12) referred to a case reported by Esmarch (21). It would appear that the diagnosis of acromegaly, although considered, was not definitely established clinically. The sella turcica was normal in size. Two islet-cell adenomas were removed surgically.

Reports of the 8 Mayo Clinic cases follow. The water test for adrenal insufficiency, referred to in Case 5, was that described by Robinson, Power and Kepler (22). Cases 7 and 8 were reported previously (23). Case 1 was included in a review of tumors of the islets of Langerhans (24).

REPORT OF CASES

Case 1. *Clinical data.*—A woman aged 34 years was first examined at the Mayo Clinic in September 1943. The family history revealed that a sister had experienced spells of confusion and had died after a week in coma. In 1932, while in the third month of pregnancy, the patient had two spells of double vision associated with a feeling of unreality or being "in a different world." The next similar spell was in 1937; thereafter they were of increasing frequency, the patient having had ten in the year before admission. At last, excessive perspiration had become prevalent during attacks, and unconsciousness had occurred twice and urinary incontinence once. On one occasion the concentration of fasting blood sugar had been 40 mg. per 100 cc. The spells occurred late in the afternoon after hard work and could be aborted by "eating sweets." She had gained 15 pounds in two years.

The physical examination disclosed moderate obesity and normal blood pressure.

TABLE 2. MULTIPLE ENDOCRINE ADENOMAS INVOLVING THE PITUITARY, PARATHYROIDS AND PANCREATIC ISLETS: MAYO CLINIC CASES

Case	First seen at clinic	Age, sex	Pituitary	Parathyroids	Pancreatic islets	Miscellaneous
1†§	1943	34 F	X-ray exam. negative	Hyperparathyroidism. One tumor removed. Persistent hyperparathyroidism	Hyperinsulinism. Single islet-cell adenoma removed. Persistent hyperinsulinism. Multiple adenomas removed. Adenomatosis of islets	
2†	1944	40 F	Chromophobe adenoma, incompletely removed	Hyperparathyroidism. Two tumors removed, 4.08 and 1.2 Gm.	Hypoglycemia (sugar, 23 mg. per 100 cc.). No exploration	
3†	1947	50 F	X-ray: intrasellar tumor. No operation	Hyperparathyroidism. Four adenomas involving three glands removed	Hypoglycemia (?). No tumor palpated at operation on stomach	Partial gastrectomy for duodenal ulcer
4†	1949	36 M	X-ray exam. negative	Hyperparathyroidism. Two adenomas removed, 10.3 and 2.6 Gm.	Hypoglycemia (sugar, 30 mg. per 100 cc.). No exploration	Metastasizing bronchial adenoma surgically removed. Duodenal ulcer
5†	1949	24 F	Acromegaly. X-ray: slight enlargement of sella	Hyperparathyroidism. Nodular hyperplasia (adenomatosis) of three removed glands	Hyperinsulinism. Five adenomas removed, largest 7 cm. in diameter	Adrenal cortical adenoma, 1.5 cm. in diameter, removed
6*	1948	28 M	X-ray: intrasellar tumor. Necropsy: large chromophobe adenoma	No clinical data. Three adenomas.	No clinical data. Multiple islet-cell tumors and adenomatosis of islets	Adenomatous nodules of adrenal cortices. Diffuse gastric polyposis. Peptic ulcer. Thyroid normal
7*†‡	1946	38 M	X-ray exam. negative. Head not examined at necropsy	No clinical data. Necropsy: three adenomatous glands, total weight 3.88 Gm.	Hyperinsulinism. Islet-cell tumor, 3.5 cm. in diameter, removed at operation. Necropsy: two tumors, 1.5 cm. and 4.0 cm. in diameter, latter cystic	Cyst, 1.5 cm. in diameter in right lobe of thyroid. Kidneys normal
8*‡	1947	54 M	No x-ray or necropsy examination of head	No serum Ca or P determinations. Right ureterolithiasis. Osteoporosis. Four adenomatous glands, total weight 2.69 Gm.	No clinical data. Multiple islet-cell tumors, metastatic to regional nodes	Diffuse gastric polyposis

* Tumors encountered at necropsy.
† Tumors encountered at operation.
‡ Reported by Rogers and co-workers (23).
§ Reported by Lopez-Kruger and Dockerty (24).

The results of neurologic examination were essentially negative except for a residual right facial palsy that had been present since 1930. The ocular fundi and perimetric fields were normal. The electroencephalogram did not disclose any abnormality. The results of roentgenographic examination of the thorax, head, lumbar portion of the spinal column and hands were negative. A plain roentgenogram and an excretory urogram did not reveal any renal calculi. The values, in milligrams per 100 cc., for fasting blood sugar were 81 and 86, for serum calcium 13.6 and 12.4, for serum inorganic phosphorus 2.5 and 2.1, and for blood urea 14 and 16; the value for serum protein was 7.7 and 7.2 Gm. per 100 cc. The amount of urinary calcium excreted was 533 mg. per twenty-four hours while the patient's diet contained 135 mg. of calcium.

The patient underwent two fasts lasting forty-eight and twenty-six hours, with blood

sugar values of 36 and 29 mg. per 100 cc., respectively. During the latter fast she exhibited unusual behavior and slight slurring of speech.

Diagnoses of hypoglycemia and primary hyperparathyroidism were made. On September 24, 1943, the pancreas was explored and a tumor 1.5 cm. in diameter removed. The patient had mild glycosuria for three days. Three weeks later she had a spell similar to those experienced before. Concentrations of blood sugar as low as 40 mg. per 100 cc. indicated persistent hypoglycemia and the presence of additional islet-cell tumors.

On readmission on March 27, 1944, the patient stated that she had continued to have spells one or two times a month, once requiring glucose intravenously to restore consciousness. The value, in milligrams per 100 cc., for blood sugar was 71, for serum calcium 12.8 and for serum inorganic phosphorus 2.6. At the end of a forty-four-hour fast the value for blood sugar was 43 mg. and she had symptoms which were promptly relieved by the injection of 50 per cent solution of glucose. The pancreas was re-explored on April 4, 1944, and the distal third was removed. There were mild hyperglycemia and glycosuria for several days. She had no recurrence of symptoms and the blood sugar was at normal levels on all subsequent visits.

The patient returned again on February 14, 1945. The level of serum calcium and serum inorganic phosphorus remained in the previous abnormal range but there was still no evidence of renal or skeletal disease. An exploratory operation on the neck was performed and a parathyroid adenoma removed. The thyroid appeared normal. During the postoperative period there was no decrease in the concentration of serum calcium, and on subsequent visits in June 1945, September 1946, and September 1947, the values for calcium and phosphorus continued in the preoperative range. When she was last seen, renal or skeletal manifestations or other clinical symptoms of the persistent hyperparathyroidism had not developed. She was advised that further exploration of the neck and possibly the mediastinum would have to be considered. She has not returned since 1947, but a letter in October 1951, indicated that she has continued in good health.

Pathologic data.—The single parathyroid tumor that was removed measured 2.5 by 1.5 by 1.5 cm. Histologically this proved to be an adenoma composed of small chief cells and transitional water-clear cells (Fig. 1*a*).

At the first operation a single tumor, 1.5 cm. in diameter, was removed from the tail of the pancreas. Histologically this was a well-encapsulated islet-cell adenoma; the cells were arranged in strands and cords and the stroma was partially hyalinized. At the second operation the distal third of the pancreas was resected. Grossly, large parts of the pancreatic tissue were replaced by a multicentric growth consisting of circumscribed rounded nodules measuring up to 1.5 cm. in diameter (Fig. 1*b*); at least eight nodules measured more than 1 cm. in diameter. The majority of the larger nodules were grouped

---

FIG. 1. (Case 1). Multiple endocrine adenomas. *a.* Histologic section of parathyroid adenoma composed of small chief cells and transitional water-clear cells. Since the concentration of blood calcium failed to decrease postoperatively, a second parathyroid tumor was believed to be present in this case (hematoxylin and eosin; ×165). *b.* Gross appearance of the distal third of the pancreas (with attached spleen), showing multiple rounded nodules on cut section. *c.* Section of the pancreas showing multiple islet-cell adenomas (hematoxylin and eosin; ×3½). *d.* Histologic section of one of the large islet-cell tumors (hematoxylin and eosin; ×185).

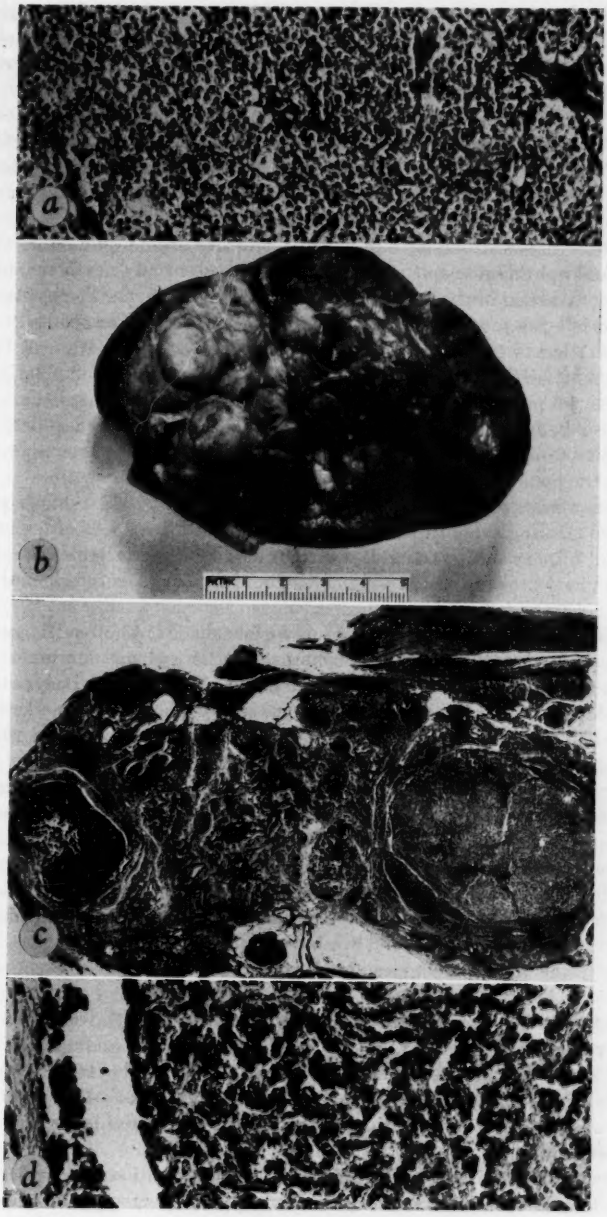


FIG. 1. *a, b, c, d.*

close together along the superior aspect of the tail of the pancreas. Histologic examination revealed islet-cell adenomatosis consisting of multiple circumscribed nodular areas ranging in size from giant islet-like clusters of cells to large islet-cell adenomas, 1.5 cm. in diameter (Fig. 1, *c* and *d*).

Case 2. *Clinical data.*—An unmarried woman, aged 40 years, was first examined at the Mayo Clinic in February 1944. Three uncles and one aunt had diabetes mellitus. The patient's menses stopped at the age of 25. About five months prior to admission she had had onset of nausea, vomiting, loss of memory and personality change. There had been no headache or visual disturbance.

Examination disclosed obesity and a blood pressure of 98 systolic and 70 diastolic. Neurologic and ophthalmoscopic examinations gave essentially negative results. There was general contraction of the perimetric fields, psychogenic type. The electroencephalogram showed left frontotemporal delta localization, grade 3. The results of roentgenoscopic examination of the stomach were negative. The roentgenogram of the head revealed erosion of the floor of the sella turcica by an intrasellar tumor. The basal metabolic rate was +5 per cent, she having discontinued taking desiccated thyroid ten days previously. On February 19, 1944, the pituitary region was explored and an intrasellar and suprasellar tumor was incompletely removed. She was given roentgen therapy to the pituitary region.

Until three weeks prior to the next admission on May 27, 1947, the patient had been generally well. During these three weeks she had four or five spells of unconsciousness, twitching and urinary incontinence. No further details were available. Except for some pallor and a blood pressure of 120/74, the results of physical, neurologic and visual field examinations were as before.

The values, in milligrams per 100 cc., were for blood sugar 43, urea 22, serum calcium 13.1, 12.7 and 12.4, and inorganic phosphorus 2.9, 2.5 and 2.2. During the period of observation, three values for blood sugar were 23, 28 and 35 respectively; others ranged from 41 to 53, and the highest was 86. The values for phosphatase and serum protein and the albumin-globulin ratio were normal. There was hypercalcinuria while the diet contained 135 mg. of calcium. The value for urinary 17-ketosteroids was 3.4 mg. per twenty-four hours. There was no evidence of osteoporosis in the roentgenograms of the thorax, skull, lumbar portion of the spinal column or hands. There was no history of renal colic and the excretory urograms did not disclose any renal calculi. An insulin-tolerance test revealed no undue sensitivity. Other than the history of amenorrhea and the pallor, there was little evidence for anterior pituitary insufficiency. Likewise there was insufficient evidence for secondary adrenal insufficiency. The basal metabolic rate was −15 per cent but the patient did not have clinical myxedema. Diagnoses of primary hyperparathyroidism and hypoglycemia were made, the latter most likely of pancreatic origin.

On June 14, 1947, a cervical exploration was performed and two enlarged parathyroid glands and one normal gland were removed. The thyroid appeared normal. By June 16, the level of serum calcium was 8.1 and by June 20, 7.0 mg. per 100 cc., and there were mild tetanic symptoms. Oral treatment with dihydrotachysterol and calcium lactate in solution was instituted. At the time of the patient's dismissal the serum calcium was at normal levels.

The patient was advised to return for further investigation and exploration of the pancreas but to date has not returned. In January 1951, correspondence indicated that her general health was good and that she was doing her usual work. Her weight was nearly 200 pounds as a result of "eating to keep my blood sugar level up to normal."

Pathologic data.—Histologic examination of tissue surgically removed from the region of the optic chiasm revealed a pituitary adenoma of chromophobe type (Fig. 2*a*).

Three parathyroid glands were completely removed and a biopsy specimen was taken from the fourth (Fig. 2*b*). Two masses of parathyroid tissue were found on the right side of the neck. The right superior gland measured 6 by 1.5 by 1 cm., was somewhat dumbbell-shaped and weighed 4.08 Gm. Histologically this mass was composed of large water-clear cells throughout, with a few transitional oxyphils (Fig. 2*c*). The right inferior gland weighed 1.2 Gm. and was composed of a mixture of large chief cells and transitional water-clear cells (Fig. 2*d*). On the left side the superior glandule was completely removed. It was greatly elongated but histologically appeared normal with wide bands of fatty stroma (Fig. 2*e*). A biopsy of the inferior gland revealed normal parathyroid tissue. In summary, then, there were two very large parathyroid tumors, the total weight being 5.28 Gm. Microscopically the larger resembled diffuse water-clear hyperplasia, the other a chief-cell adenoma. The remaining two glands were normal.

Case 3. *Clinical data.*—A woman, aged 50 years, came to the Mayo Clinic on March 21, 1947, primarily because of severe symptoms of peptic ulcer. One sister and her father had had peptic ulcer. The patient's menstrual history was normal but she had never been pregnant. For two years she had had intermittent symptoms compatible with those of a duodenal ulcer. For twenty-three years she had had spells consisting of weakness, perspiration, blurring of vision, tremor, disorientation and amnesia. There had been four periods of unconsciousness between 1929 and 1943. These, as well as the other spells, had always been relieved by dextrose taken orally. She had taken 6 heaping teaspoons of dextrose daily for twenty years to avoid spells. On one occasion, about 1932, the value for blood sugar had been 36 mg. per 100 cc.

The patient was short (58¼ inches) and of average weight. She had mild hirsutism. The hands were deformed by Heberden's nodes and the feet by bunions. There were also slight suggestive acromegalic changes of the hands but other substantiating evidence was lacking. There was some nodularity of the thyroid gland. The blood pressure was 152/100. The results of neurologic examination were negative and the perimetric fields were normal. The values, in milligrams per 100 cc., were for fasting blood sugar 70, blood urea 69 (after gastric operation it was down to 40), serum calcium 13.8, 12.5 and 12.3, and serum inorganic phosphorus 2.2, 2.4 and 2.0. The value for serum protein and the albumin-globulin ratio were normal. She had hypercalcinuria (290 and 358 mg. of calcium per twenty-four hours) while the diet contained 135 mg. of calcium. The value for gastric acids was 130 units for total acid and 114 units for free hydrochloric acid. The urinary excretion of 17-ketosteroids measured 9.2 mg. and of corticosteroids, 0 4 mg. per twenty-four hours. The basal metabolic rate was +22 per cent. A duodenal ulcer was demonstrated on roentgenoscopic examination. Roentgen examination of the head revealed marked enlargement of the sella turcica by an intrasellar tumor. Roentgenograms of the hands showed osteoarthritic changes but no osteoporosis or tufting. No renal calculi were seen on a plain roentgenogram. Two forty-eight-hour fasts failed to produce hypoglycemic symptoms and the concentrations of blood sugar were 90 and 86 mg. per 100 cc., respectively. During the stay in the hospital, however, values of 56, 60 and 63 mg. were obtained.

Diagnoses were made of chronic duodenal ulcer with obstruction, pituitary tumor and primary hyperparathyroidism without renal or skeletal disease. The history was highly suggestive of hypoglycemia.

Because the patient was not relieved of her severe ulcer symptoms by a medical regimen, subtotal gastrectomy was carried out on April 9, 1947. During the course of

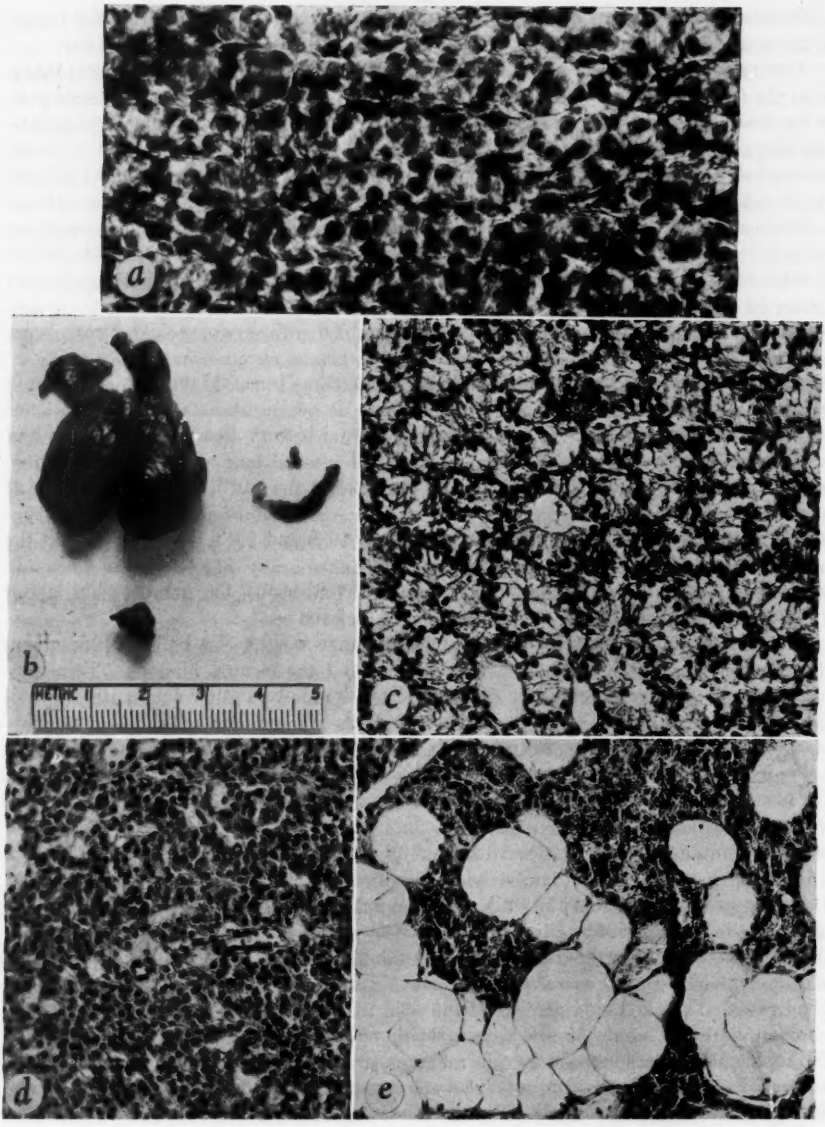


FIG. 2 (Case 2). Multiple endocrine tumors. *a.* Histologic section of a chromophobe adenoma of the pituitary (hematoxylin and eosin; ×320). *b.* Gross appearance of parathyroid tissue removed surgically. The largest mass of tissue (right superior gland) was dumbbell-shaped before removal. Only a portion of the right inferior gland is shown. *c.* Histologic section of the large right superior gland, showing large water-clear cells (hematoxylin and eosin; ×165). *d.* Chief-cell adenoma of the right inferior gland (hematoxylin and eosin; ×165). *e.* Histologic section of the normal elongated left superior glandule (hematoxylin and eosin; ×165).

this operation no tumor was palpated in the pancreas and no pancreatic tissue was removed.

On June 3, 1947, subtotal thyroidectomy was carried out and three enlarged parathyroid glands were also removed. By June 7, 1947, the level of serum calcium was 8.4 and of serum inorganic phosphorus, 3.3 mg. per 100 cc.; the urinary excretion of calcium was down to 24 mg. per twenty-four hours. No tetany developed.

The patient was re-examined in September 1947, at which time she stated she had had three more episodes of confusion. The value for blood sugar was 60, for serum calcium 9.4, and for serum inorganic phosphorus 3.5 mg. per 100 cc. The basal metabolic rate was +3 per cent. In the perimetric fields there was a slight but definite bitemporal hemianopsia which had progressed when rechecked four weeks later. She was given

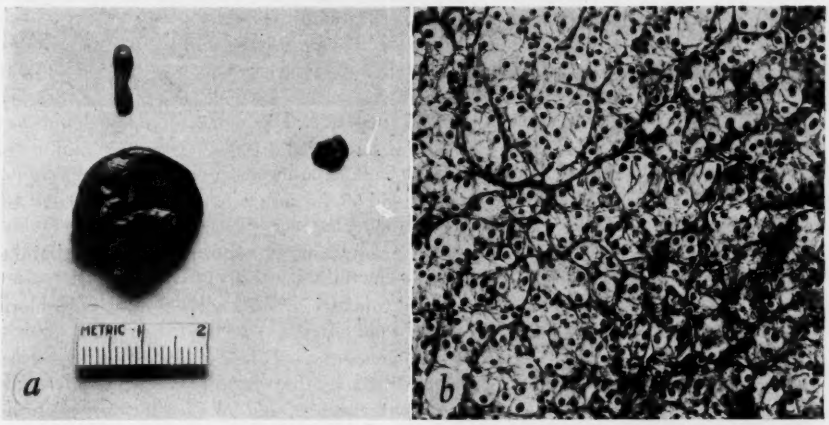


FIG. 3 (Case 3). Multiple endocrine tumors. *a.* Multiple adenomas of the parathyroid glands removed surgically. *b.* Histologic section of the largest adenoma, showing a mixture of large water-clear and oxyphil cells (hematoxylin and eosin; ×165).

roentgen therapy to the pituitary region. On December 29, 1947, the perimetric fields had returned to normal. Since then they have remained normal, having been examined every six to twelve months, the last time being on May 15, 1952.

The patient has continued to have occasional periods of confusion and once was unconscious in the early morning. Because she lives several miles from a town, there unfortunately has never been an opportunity to get a determination of blood sugar during an attack. We have continued of the opinion that most likely her symptoms are on the basis of hypoglycemia and are due to an islet-cell lesion, even though no tumor was palpated at the time of gastric operation.

Pathologic data.—Gross examination of the portion of stomach removed because of duodenal ulcer revealed hypertrophic gastritis with greatly enlarged tortuous rugae.

The 12 Gm. of thyroid tissue removed surgically was found on histologic examination to be essentially normal, with large colloid-filled vesicles. Of the three enlarged parathyroids removed surgically (Fig. 3*a*), the right inferior measured 2 cm. in diameter and weighed 1.9 Gm.; histologically this adenoma was composed of large water-clear cells and oxyphil cells in approximately equal proportions with numerous transition forms (Fig. 3*b*). The right superior glandule weighed 150 mg. and was composed of one small oxyphil-cell and one chief-cell adenoma. The adenoma removed from the left

side weighed 140 mg. and was composed entirely of small chief cells. Thus, there were four parathyroid adenomas involving three glands.

Case 4. *Clinical data.*—A man, aged 36 years, was first seen at the Mayo Clinic on March 1, 1949. His father had died during an operation for peptic ulcer and the patient's twin brother, who had renal calculi, was found at necropsy to have carcinoma of the tail of the pancreas. In February 1948, he had been found to have, on bronchoscopic examination, a lesion of the right lower bronchus. The lesion removed at lobectomy elsewhere had proved pathologically to be bronchial adenoma with metastasis to a regional node. About September 1948, the patient had had onset of stiffness, weakness, dull aching, and a tired feeling in the anterior part of both thighs. There had been some polyuria and polydipsia for about a year.

Examination on admission revealed changes compatible with those of thickened pleura over the right lower posterior part of the thorax. There was tenderness to palpation of both thighs, and bilateral weakness of the quadriceps and hamstring muscles. The deep reflexes were hyperactive but equal. The blood pressure was 122/70 and the results of ophthalmoscopic examination were negative.

The results of urinalysis were essentially negative. The value for hemoglobin was 9.4 Gm. per 100 cc. of blood, the erythrocytes numbered 3,100,000 per cubic millimeter and the blood smear showed hypochromic anemia. The level, on different occasions, for blood urea was 44 and 28, for blood sugar 32 and 56, for serum calcium 15.3, 15.9 and 16, and for serum inorganic phosphorus 3.2, 3.3 and 3.4 mg. per 100 cc. The value for alkaline phosphatase was 38 Bodansky units. The concentration of serum protein, the albumin-globulin ratio and the results of the sulfobromophthalein test for liver function were normal. Gastric analysis revealed 104 units of total acid and 74 units of free hydrochloric acid. Roentgenograms of the thorax disclosed old surgical defects of the ribs and thickened pleura at the right base. Multiple roentgenograms of the skeleton revealed typical manifestations of skeletal abnormalities caused by hyperparathyroidism. The sella turcica did not appear enlarged. A plain roentgenogram and an excretory urogram did not show the presence of renal calculi.

Other than one episode, in September 1948, of weakness and hunger relieved by food, there were no suggestive symptoms of hypoglycemia. After the low morning levels of blood sugar were obtained, the patient underwent a fast and after sixteen hours had repeated vomiting which was promptly relieved by the intravenous injection of glucose. The concentration of blood sugar was 34 mg. per 100 cc. The following day another fast was started and by 6 p.m. the patient was mentally confused and exhibited a drunken behavior. He had prompt mental clearing after 25 cc. of 50 per cent solution of glucose had been injected intravenously, except that there was amnesia from 3 to 6 p.m. The value for blood sugar was 30 mg. per 100 cc. Diagnoses were made of primary hyperparathyroidism with severe generalized osteitis fibrosa cystica and hypoglycemia probably due to islet-cell tumor.

Exploration of the parathyroids was carried out on March 12, 1949, and two enlarged glands were removed. On the following day the concentration of serum calcium had fallen to 9.2 and by March 21 it had decreased to 5 mg. per 100 cc., in spite of the fact that large oral doses of calcium lactate in solution had been started on March 17. As had been anticipated because of the severe bone disease, he had symptoms of tetany which for several weeks required a tremendous amount of treatment. As much as 32 Gm. of calcium gluconate given intravenously, 2 drams of calcium gluconate by mouth every two hours (while awake) and 250,000 units of vitamin D were required daily.

Unfortunately, on April 4, 1949, a door swung against him and he fell, sustaining

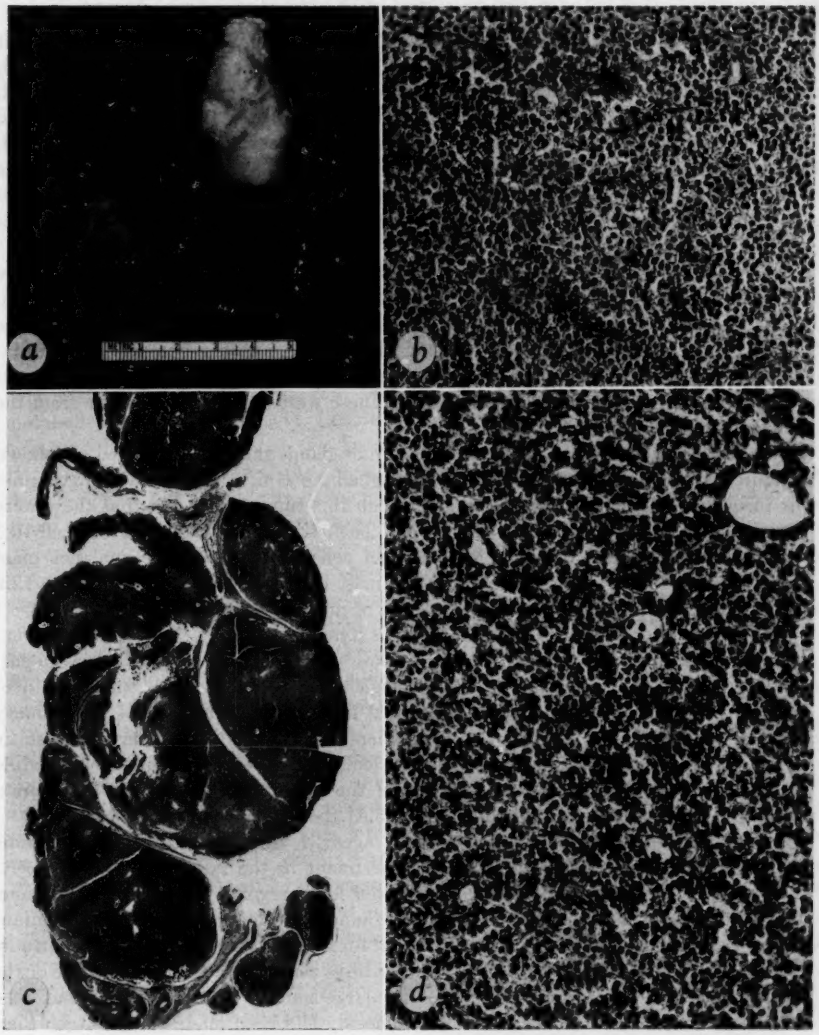


FIG. 4 (Case 4). Multiple endocrine tumors. Parathyroid glands. *a.* Gross appearance of two large adenomas surgically removed. *b.* Histologic section of the right lower parathyroid, showing chief-cell adenoma (hematoxylin and eosin; ×165). *c.* Low-power view of this tumor, to show multiple adenomatous foci of growth (hematoxylin and eosin; ×3½). *d.* Histologic section of the left superior parathyroid, showing chief-cell adenoma (hematoxylin and eosin; ×165).

fractures of the proximal end of the right humerus and the left femur. Appropriate orthopedic treatment was instituted.

On June 9, 1949, after a fast of fifty hours he was excitable and had odd behavior and the value for blood sugar was 38 mg. per 100 cc. Because of the pulmonary lesion, recent operation and multiple fractures, exploration of the pancreas was not advised.

The patient has continued to have occasional hypoglycemic episodes and, although he has been re-examined four times, operation on the pancreas has not been performed. He continued taking a moderate amount of calcium gluconate for about a year but since then the values for serum calcium and serum inorganic phosphorus have remained normal without treatment. Roentgenograms of the skeleton have shown marked recalcification.

When the patient was examined in November 1950, he had moderately severe dyspepsia, and a duodenal ulcer was demonstrated on roentgenoscopic examination. He has had three metastatic lesions removed: one from the right cervical region at the time of the operation on the parathyroids in March 1949, another from the right supraclavicular region in November 1950, and the last, a subcutaneous nodule from the upper anterior wall of the thorax, in January 1952. These were all considered to be from the bronchial adenoma.

Pathologic data.—The two enlarged parathyroid glands that were removed are shown in Figure 4*a*. The right lower parathyroid weighed 2.6 Gm. and was made up of chief cells throughout (Fig. 4*b*). A cut section through this tumor suggested that there were multiple adenomatous foci of growth (Fig. 4*c*). The left upper parathyroid weighed 10.3 Gm. and was composed of small regular chief cells forming a single compact mass (Fig. 4*d*). In summary, there were two adenomas, the total weight of which was 12.9 Gm. In one gland the structure was suggestive of multiple foci of growth.

At the time of operation on the parathyroids a metastatic mass, 3 by 2.5 by 1.5 cm., was found in the right upper cervical region, closely adherent to the esophagus and surrounding structures. It was found, on histologic examination, to be a small-cell neoplasm with occasional mitotic figures, identical in structure with that of the primary bronchial lesion removed at lobectomy elsewhere. At a second operation a metastatic nodule was removed from the right supraclavicular region and subsequently a nodule was removed from the subcutaneous tissue of the upper anterior part of the thorax. Histologically these metastatic nodules resembled the primary bronchial lesion.

Case 5. *Clinical data.*—A married woman, aged 24, first came to the Mayo Clinic on December 12, 1949. There was nothing pertinent in the family history. In 1944, her menses had become irregular and in 1946 her first pregnancy terminated spontaneously at three and a half months. Her next pregnancy resulted in a normal female infant delivered in January 1948. She nursed the infant for three months but did not menstruate again. During the summer of 1948 she and her physician had noted enlargement of the hands, feet and facial features. Evidence of enlargement of the sella turcica had been seen on a roentgenogram. A diagnosis of acromegaly had been made and roentgen therapy to the pituitary region had been given.

Examination here disclosed obesity (weight, 181 pounds) and characteristic acromegalic changes, such as prominent nose, protruding chin, large tongue, some separation of the incisor teeth, large spade-shaped hands, large broad feet and a heavy trunk. The blood pressure was 120/75. The results of neurologic and ophthalmoscopic examinations were negative. In the perimetric fields there was minimal but definite bitemporal hemianopsia. The value, in milligrams per 100 cc., for blood sugar was 66, for serum calcium 11.4 and 10.8, and for serum inorganic phosphorus 3.9 and 4.1; the concentra-

tion of serum protein was 5.8 Gm. per 100 cc. Urinary 17-ketosteroids measured 4.4 mg. per twenty-four hours and the basal metabolic rate +17 per cent. There was minimal enlargement of the sella turcica according to the roentgenogram. Further roentgen therapy to the pituitary body was advised and this was administered by a roentgenologist in her home community.

The patient returned on November 17, 1950, having noted no further progression of the acromegalic features. A month prior to return she had two episodes of confusion in the morning before breakfast, which were relieved after eating. Further history revealed that she had had two or three similar episodes in 1946. There had been no convulsions.

The results of examination were essentially as before, except that her weight was 194 pounds and the perimetric fields were nearly normal. The level of blood sugar was 56, blood urea 14, serum calcium 12 and 12 and serum inorganic phosphorus 3.3 and 3.8 mg. per 100 cc. The value for serum protein was 5.8 Gm. and for alkaline phosphatase 2.2 Bodansky units. The urinary excretion of calcium, while the diet contained 135 mg. of calcium, was 375 and 228 mg. per twenty-four hours. The value for urinary 17-ketosteroids was 8.1 and for corticosteroids 0.76 mg. per twenty-four hours (22). The basal metabolic rate was +7 per cent. Results of the water test for adrenal insufficiency were negative. Roentgenograms of the head revealed that no change had occurred since December 1949, and those of the hands disclosed no change in respect to hyperparathyroidism. There was no evidence of calcification in the plain roentgenogram of the kidney, ureter and bladder, or in the excretory urogram.

During examination two episodes of confusion and disorientation occurred, one being associated with a convulsion in the early morning. These were relieved after eating. She was admitted to the hospital for a fast; after eight and a half hours she was confused and disoriented and the concentration of blood sugar was 38 mg. per 100 cc. There was prompt relief of her symptoms in three to five minutes after the injection of 20 cc. of 20 per cent solution of glucose. In addition to acromegaly, we made diagnoses of primary hyperparathyroidism and hypoglycemia probably due to an islet-cell lesion.

On November 28, 1950, the pancreas was explored and multiple tumors were removed. Palpation of the adrenals revealed also a left adrenocortical tumor which was removed. Hyperglycemia and glycosuria developed postoperatively to a degree sufficient to require administration of up to 16 units of insulin a day for three weeks, but none thereafter. On subsequent visits the concentration of fasting blood sugar ranged between 109 and 118 mg. per 100 cc. and on March 17, 1952, a glucose-tolerance curve was normal. There was no recurrence of hypoglycemic episodes.

The patient returned again and underwent cervical exploration on January 11, 1951; three enlarged parathyroid glands were removed. On the following day symptoms and signs of tetany developed and the value for serum calcium had dropped to 8 mg. per 100 cc. The symptoms were controlled with dihydrotachysterol and calcium lactate.

The patient was seen on three occasions after the cervical operation, the last time in March 1952. She was in good health and had no headaches. By dieting she had reduced her weight to 157 pounds. There had been a little decrease in glove and shoe sizes, probably as a result of reduction in weight. The perimetric fields were normal. The urinary 17-ketosteroids measured 6.1 mg. per twenty-four hours, the basal metabolic rate was −9 per cent, the value for serum calcium was 9.8 mg. and the value for serum inorganic phosphorus was 3.9 mg. per 100 cc.

Pathologic data.—The three enlarged parathyroid glands are shown in Figure 5*a*. The left superior gland weighed 200 mg. and was completely removed. Histologically

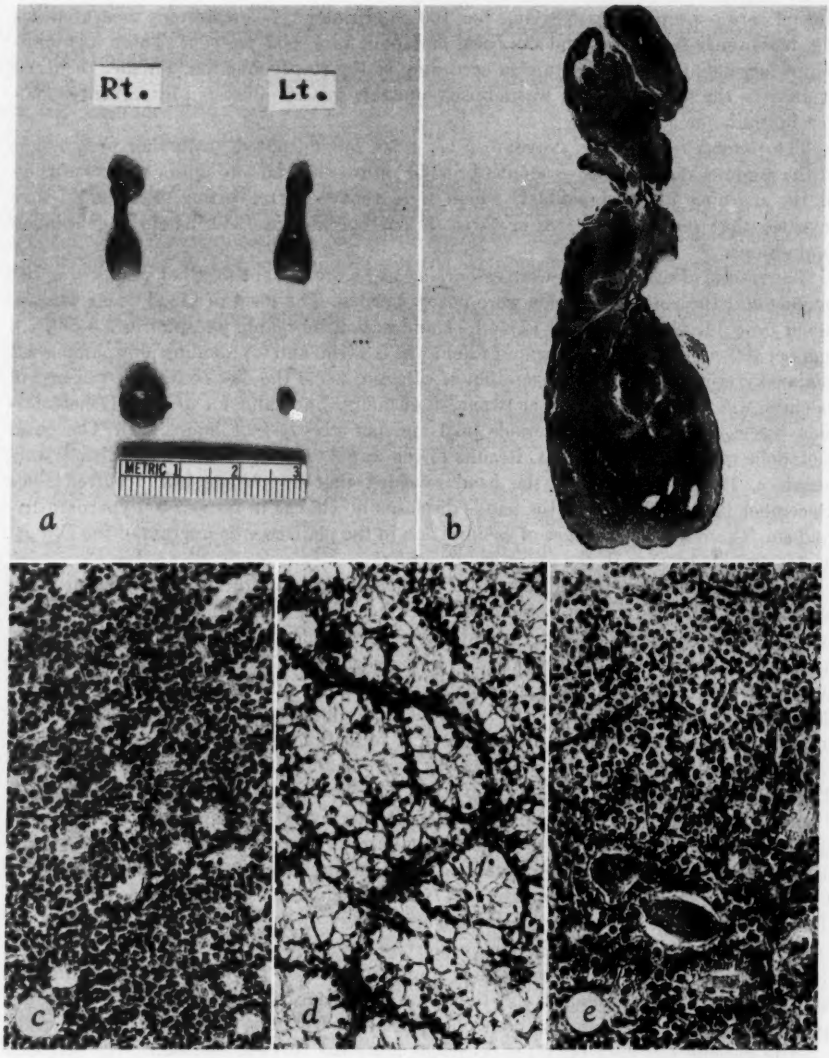


FIG. 5 (Case 5). Multiple endocrine tumors. *a.* Gross appearance of parathyroid glands surgically removed. Note nodularity of the right and left superior glands. *b.* Low-power view of the left superior gland, suggesting nodular hyperplasia (hematoxylin and eosin; ×3). *c.* Largest nodule in the left superior gland, composed of chief cells in a glandular pattern (hematoxylin and eosin; ×200). *d.* Corresponding nodule in the right superior gland, composed of large water-clear cells (hematoxylin and eosin; ×200). *e.* Histologic section of the right inferior gland, showing large chief cells with some acinar formation (hematoxylin and eosin; ×200).

there were multiple adenomatous foci. The largest of these was composed of chief cells (Fig. 5*b* and *c*). The smaller nodules were made up of transitional water-clear cells and oxyphil cells. The right superior gland was irregular in shape with pseudopod formation. A subtotal resection was carried out, removing 200 mg. of tissue and leaving approximately 40 mg. Histologically this gland was composed of multiple adenomatous nodules, the largest 0.9 cm. in diameter, made up of water-clear cells (Fig. 5*d*); the re-

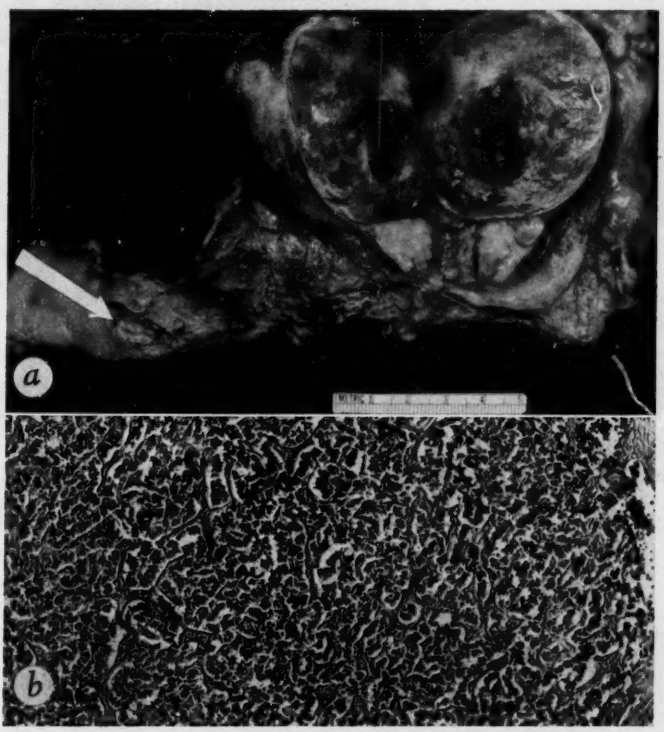


FIG. 6. (Case 5). *a*. Gross appearance of resected body and tail of pancreas showing one very large, and multiple small islet-cell tumors. *b*. Histologic section of the small islet-cell adenoma indicated by the arrow on the gross specimen (hematoxylin and eosin; ×90).

maining nodules in this gland consisted of chief and oxyphil cells. The right inferior gland weighed 300 mg. and was completely removed. It had been replaced by a single compact adenoma of large chief cells with a somewhat glandular pattern (Fig. 5*e*). From the left inferior position, 20 mg. of parathyroid tissue was removed. No further parathyroid tissue was found in this region. In summary, there was nodular hyperplasia (adenomatosis) of three parathyroid glands (total weight, approximately 740 mg.), the remaining gland not being involved.

Gross examination of the pancreas revealed five tumors ranging in size from 5 mm. to 7 cm. in diameter (Fig. 6*a*). The largest, situated in the tail, was encapsulated and

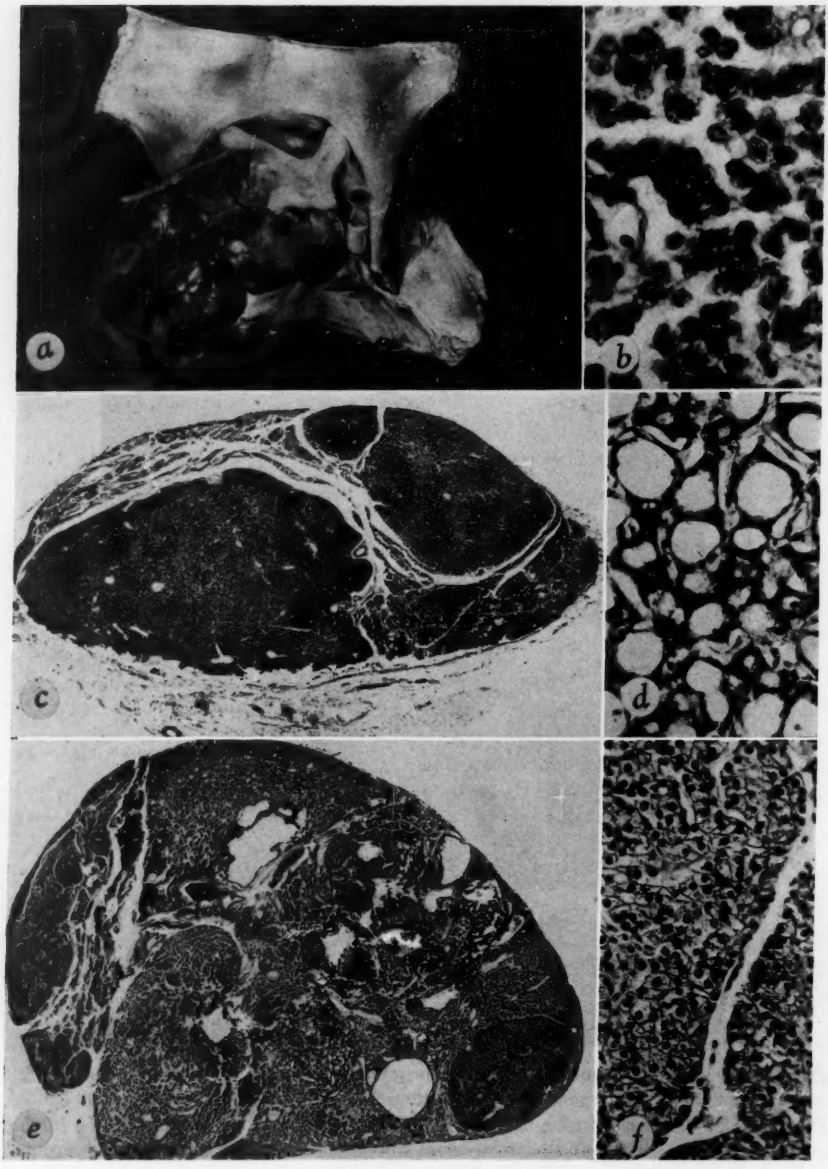


FIG. 7 (Case 6). Multiple endocrine adenomas. *a*. Gross appearance of pituitary tumor displacing optic chiasm. *b*. Histologic section showing typical chromophobe adenoma (hematoxylin and eosin; ×415). *c*. Low-power view of the right superior parathyroid, showing adenomatous nodules (hematoxylin and eosin; ×8). *d*. Histologic section of

lobulated. Of the remaining tumors, two were situated in the body of the pancreas and two in the tail. Microscopically the tumors were typical islet-cell adenomas composed of small cells arranged in cords, strands or ribbons. A delicate highly vascularized stroma was present (Fig. 6*b*).

A single adrenal cortical adenoma, 1.5 cm. in diameter, was surgically removed.

Case 6. *Clinical data.*—An unmarried man, aged 28, came to the Mayo Clinic on July 1, 1948, chiefly because of obesity. There was a striking paternal family history: the father, who had had three operations for duodenal ulcer, died at the age of 42; two uncles weighing 340 and 252 pounds, respectively, died at the age of 42 of brain tumor and another uncle, who weighed 200 pounds, died at the age of 52 of brain tumor (acromegaly?); one sister, aged 23, was "getting fat" and "pancreatic tumor" had been suspected.

The patient had always been a heavy eater and weighed 180 pounds at the age of 16, 265 at the age of 20 and more than 300 pounds when examined. He had been found to have a pituitary tumor in 1940, but had had no treatment. He had no headache or symptoms suggestive of hypoglycemia. He had always had a thin beard and little libido. He had taken a grain of desiccated thyroid daily for two months.

Examination revealed marked obesity (weight, 307 pounds; height, 70½ inches). The blood pressure was 140/80. There were no features of acromegaly. He had a sallow appearance, a scant beard, and a moderate amount of hair in the axillary and pubic regions and on the extremities. The testes were somewhat smaller than normal. The results of neurologic and ophthalmoscopic examination were negative. Examination of the perimetric fields disclosed a right incongruous homonymous hemianopsia interpreted as being due to involvement of the left optic tract.

Roentgenograms of the head showed erosion of the floor and dorsum of the sella by an intrasellar tumor with suprasellar extension. The value for hemoglobin and the erythrocyte count were within normal limits. The value for fasting blood sugar was 83 mg. and for cholesterol it was 117 mg. per 100 cc. The urinary 17-ketosteroids amounted to 8.1 and corticosteroids to 0.9 mg. per twenty-four hours. The basal metabolic rate was −16 per cent while desiccated thyroid was being taken.

Because of the defect of the visual fields he was advised to have surgical removal of the pituitary tumor but he refused. He was given a weight-reducing diet. Although his appearance suggested anterior pituitary insufficiency, that diagnosis could not be substantiated.

The patient returned again on June 1, 1950, having had symptoms typical of peptic ulcer for six months and a roentgenoscopic diagnosis of duodenal ulcer. The results of examination were essentially as before, the weight then being 296 pounds. There was some increase of the defect of the visual fields but the defect continued to be of the incongruous right homonymous type. Roentgenoscopic examination of the stomach was reported as showing a duodenal ulcer. The urinary 17-ketosteroids measured 1.7 and the corticosteroids 0.53 mg. Because of continued pain, vomiting and gastric retention, an operation was carried out on June 14, 1950. A large inflammatory mass was found in the region of the jejunum, and gastroenterostomy and enteroenterostomy were performed. Because of possible secondary adrenal insufficiency, as suggested by the lowered

---

adenoma in the region of the right inferior parathyroid. Note chief cells with marked glandular pattern (hematoxylin and eosin; ×165). *e.* Low-power view of the left inferior parathyroid (hematoxylin and eosin; ×6½). *f.* Histologic section of this adenoma, showing transitional water-clear cells (hematoxylin and eosin; ×165).

value for urinary 17-ketosteroids, he was given aqueous adrenal cortical extract during the postoperative period. Roentgen therapy was administered to the pituitary region.

The patient's next visit, in July 1951, was made because of severe vomiting and gastric hemorrhage that had required numerous transfusions. Following treatment of severe hypochloremic hypokaliemic alkalosis he underwent a further gastro-intestinal operation. Despite a tremendous exchange of fluid and electrolyte, he was progressing satisfactorily until he had a massive hemorrhage on the fourteenth postoperative day, when death occurred within a few minutes. There had been no symptoms of hypoglycemia or hyperparathyroidism, and no determinations of serum calcium or phosphorus had been made.

Pathologic data.—The anatomic diagnoses made at necropsy were as follows: 1) previous (fourteen months) anterior gastroenterostomy and enteroenterostomy, 2) former (fourteen days) discontinuance of gastrojejunostomy, closure of opening of the stomach, jejunojejunostomy, and closure of perforating gastroesophageal ulcer, 3) ulcerative esophagitis with ulceration of the esophagogastric junction and hemorrhage (500 cc.), 4) focal peritonitis in the region of the perforation, 5) polyposis of the stomach, 6) chromophobe adenoma of the pituitary with extension into the left temporal lobe, 7) multiple parathyroid adenomas, 8) multiple adenomas of the islets of Langerhans, 9) nodular hyperplasia of the adrenal cortices, 10) atrophy of the thyroid and testes and 11) pulmonary edema with bilateral hydrothorax. Details of the lesions pertinent to this discussion follow.

The sella turcica contained a tumor, soft in consistency and dark red in color, which extended out of the sella caudally and upward into the temporal lobe. The extrasellar portion of the tumor appeared to be encapsulated and measured 4 by 2.5 by 2.5 cm. The sella turcica and its contained tumor are shown in Figure 7*a*. Histologically this proved to be an adenoma of chromophobe type (Fig. 7*b*).

Three enlarged parathyroids were identified; these measured 1.3 by 1.0 by 1.0 cm., 1.0 by 0.8 by 0.8 cm. and 1.2 by 0.5 by 0.5 cm., respectively. Weights were not obtained. The right superior parathyroid (Fig. 7*c*) contained two adenomatous nodules, one composed of chief and one of oxyphil cells. In the position of the right inferior gland there was an adenoma composed largely of chief cells with a pronounced glandular pattern (Fig. 7*d*). A few small foci of oxyphils were present. The left inferior gland was replaced by an adenoma, the low-power view of which is shown in Figure 7*e*. Some nodularity was noted on cross section. Histologically this adenoma was composed of transitional water-clear cells (Fig. 7*f*) and chief cells in glandular pattern. No oxyphils were seen. A fourth gland was not identified.

Gross examination revealed several rounded nodules in the head of the pancreas, the largest of which was cystic and measured 2 cm. in diameter (Fig. 8*a*). Histologic section disclosed three well-encapsulated islet-cell tumors measuring 0.8, 1.2 and 2 cm. in diameter, respectively. In addition to these large tumors, there was diffuse adenomatosis of the insular tissue throughout the pancreas, the nodules varying in size from greatly enlarged islets to the large tumors seen grossly on cut section (Fig. 8*b* and *c*).

⟹

FIG. 8. (Case 6). Pancreas. *a*. Gross appearance, showing multiple islet-cell tumors in the head of the pancreas, one with central cystic degeneration. *b*. Low-power view of a small islet-cell tumor (hematoxylin and eosin; ×32). *c*. Histologic section of a larger islet-cell tumor (hematoxylin and eosin; ×125). *d*. Cut surface of adrenals to show nodularity of cortex. *e*. Low-power view of adrenal cortical adenoma (hematoxylin and eosin; ×5).

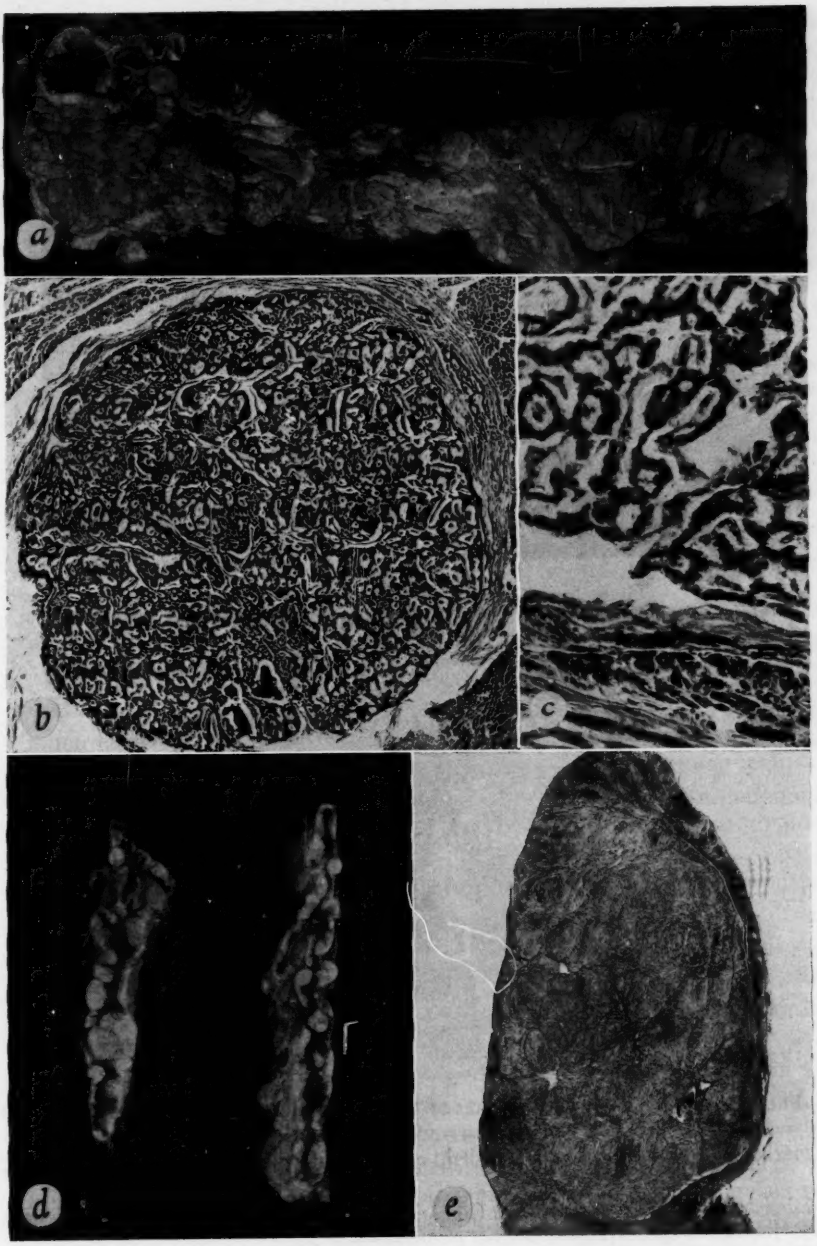


FIG. 8. *a, b, c, d, e.*

FIG. 9 (Case 6). *a*. Gross appearance of the stomach, showing diffuse polyposis (Menetrier's disease). *b*. Low-power view of cross section of one very large ruga. There is marked glandular hyperplasia and slight cystic change (hematoxylin and eosin; ×3½).

Sections of the tumors stained with Gömöri islet-cell stain showed alpha and beta cells to be present in approximately normal proportions.

Both adrenals contained multiple adenomatous nodules, the largest of which was 1.5 cm. in diameter. The gross appearance of the adrenals and low-power view of one of the adenomatous nodules are shown in Figure 8*d* and *e*.

In the cardiac region of the stomach there was a perforating peptic ulcer, 1 cm. in diameter. Distal to this and involving approximately three fourths of the stomach there was diffuse polyposis of the gastric mucosa with giant rugae characteristic of Menetrier's disease (Fig. 9*a* and *b*). In the second part of the duodenum there was a chronic ulcer that measured 1.5 by 1 cm.

Cases 7 and 8.—(These cases have been previously reported by Rogers and coworkers [23].) In Case 7 there was clinical hyperinsulinism with surgical removal of an islet-cell tumor 3.5 cm. in diameter. Death occurred eight days postoperatively and at necropsy two additional islet-cell tumors were found. Three adenomatous parathyroid glands were found, measuring 3.2 by 2 by 1, 1.8 by 1 by 1, and 1 by 0.8 by 1 cm., respectively. Serum calcium, serum inorganic phosphorus or blood urea levels were not determined preoperatively. The kidneys were normal at necropsy, and on this basis it was felt that parathyroid hyperplasia secondary to renal disease could be excluded.

In Case 8 there were right ureterolithiasis and osteoporosis. Unfortunately, determinations of serum calcium and phosphorus were not made. There was no history of hypoglycemic episodes and the concentration of blood sugar was not determined. The patient died two days after ureterolithotomy. At necropsy there were multiple adenomas of all four parathyroid glands, which measured 2.2 by 0.9 by 0.5, 2.5 by 1 by 0.7, 2 by 1.3 by 1, and 1.4 by 0.9 by 0.6 cm., respectively. The pancreas contained multiple islet-cell tumors, with metastasis to regional lymph nodes. There was diffuse polyposis of the stomach.

COMMENT

A comparison of our series of multiple endocrine adenomas with the series from the literature shows marked similarity in pathologic findings in the parathyroids and pancreas (Tables 1 and 2). In the series from the literature (Table 1) there is a greater incidence of pituitary tumors and a much larger proportion of patients with acromegaly than in our series.

Pituitary.—In our 8 cases the positive findings in the pituitary included two chromophobe adenomas (one surgical and one necropsy), an adenoma associated with clinical acromegaly, and an intrasellar tumor demonstrated on roentgenograms. Of the remaining 4 cases, in 3 there was no demonstrable enlargement of the sella turcica by roentgen examination and in the fourth neither roentgenologic nor necropsy examination of the sella was made. There was a pituitary tumor in all but 1 of the 14 cases from the literature; in 9 of these there was acromegaly, in 3 there were chromophobe adenomas and in 1 an intrasellar tumor of indeterminate variety was disclosed on roentgenographic examination.

Parathyroids.—In all of our cases there were multiple parathyroid tumors except in Case 1, in which removal of a single parathyroid adenoma did not result in a decrease in concentration of the serum calcium. This persistent hyperparathyroidism probably indicates the presence of an additional parathyroid tumor or tumors but further exploration has not yet been attempted. In addition to involvement of multiple parathyroid glands there were multiple adenomatous foci in individual glands in some cases; this we have termed "adenomatosis" or "nodular hyperplasia" of the parathyroids.

Parathyroid tumors were present in 12 of the 14 cases in the literature. In 5 of these, multiple tumors were encountered at necropsy and in another a single adenoma and three "hyperplastic glands" were observed. In 2 additional necropsy cases there were single large parathyroid adenomas; in 1 of these cases no additional parathyroids were found and in the other no mention was made of the remaining glands. Of the 4 cases in which single parathyroid tumors were removed surgically, in 1 the patient was cured, in 1 hypercalcemia and hypophosphatemia persisted and in the remaining 2 no postoperative data were given in the case reports.

Pancreas.—Five of our patients had islet-cell tumors, proved at operation or necropsy. Since 2 of the remaining 3 patients were found to have severe hypoglycemia fulfilling Whipple's triad, it was felt that the hypoglycemia in these cases was probably due to islet-cell tumors, since other organic causes of hypoglycemia were excluded. The third patient had a history strongly suggestive of hypoglycemia but this was not demonstrated objectively. The fact that a tumor of the pancreas was not palpated at the time of operation for ulcer in this case does not exclude the presence of a hyperfunctioning islet-cell adenoma since, as is well known, small adenomas may be difficult or impossible to palpate.

Just as multiple tumors were encountered in the parathyroids, so was multiplicity observed in all 5 cases of islet-cell tumor, and in addition diffuse adenomatosis of the pancreatic islets was noted in 2 cases (Cases 1 and 6).

In the series from the literature (Table 1) 8 patients had islet-cell tumors, and of these, 6 had multiple islet-cell tumors, 1 a single adenoma with hypertrophied islets and 1 a single hyperfunctioning islet-cell tumor which was removed at operation. No postoperative data were given in this latter case.

Multiplicity.—In spite of the multiplicity of tumors or islet-cell adenomatosis, the hypoglycemia in our patients was no different from that in the usual case of single islet-cell adenoma. In 2 of our necropsy cases in which there were multiple islet-cell tumors (in 1 with metastasis), however, no clinical symptoms of hypoglycemia were presented.

Despite the fact that parathyroid tumors were present in all 8 of our cases, in only 1 surgical and 1 necropsy case was there renal or skeletal disease. In all 5 surgical cases there was proved primary hyperparathyroidism with positive chemical findings; however, in only Case 4 were conspicuous clinical manifestations of the disease present. In 4 surgical cases there were no discernible renal or skeletal involvement and no clinical symptoms attributable to parathyroid disease. This is most surprising when compared to the findings in patients with renal manifestations sometimes associated with a very small single hyperfunctioning adenoma. The pa-

tient in Case 1 has been known to have persistent hyperparathyroidism since 1943, with a value of more than 12 mg. per 100 cc. for serum calcium, and yet correspondence in 1951 indicated that she was in good health and had no symptoms referable to skeletal or renal disease.

It is of interest to note that in these cases parathyroid disease was found either accidentally or because we suspected it owing to the presence of associated pancreatic or pituitary disease. Despite the absence of clinical symptoms or complications of hyperparathyroidism in these instances, its existence was thoroughly established by 1) the finding of characteristic chemical changes in the blood, 2) the finding of parathyroid lesions when they were sought, and 3) the correction of the chemical abnormalities upon removal of the lesions, except in Case 1. In at least 2 instances in Mayo Clinic experience, in addition to the cases in this report, hyperparathyroidism has been accidentally found and similarly proved, despite absence of any renal or skeletal manifestations or any general symptoms referable to the condition. Such cases suggest that hyperparathyroidism may exist without any clinical manifestations or symptoms which call attention to it.

In Case 5 the presence of a high normal or slightly elevated value for serum inorganic phosphorus on different occasions may possibly have been the resultant of the tendency to hyperphosphatemia due to the acromegaly and the hypophosphatemia due to the hyperparathyroidism.

The occurrence of multiple parathyroid adenomas is rare. Norris (25) in a collective review of the literature in 1947 found only 20 examples in 322 cases of parathyroid adenoma. In the total Mayo Clinic experience with hyperparathyroidism due to adenoma (126 cases), 11 examples of multiple adenomas were reported (26), including the 5 described herein.

Multiple islet-cell tumors are also uncommon. Crain and Thorn (27), in a collective review of 258 cases of functioning pancreatic islet-cell adenomas, found that more than one tumor was present in 12 per cent of the cases in the benign group. Diffuse adenomatosis of the islets associated with islet-cell adenomas is an extremely rare condition. Excluding the cases in our series, we know of only 4 examples of this condition (28–30).

The occurrence of multiple adenomas or adenomatosis, both in the parathyroids and in the pancreatic islets, in the cases of the syndrome under discussion in this paper, is almost the rule. Therefore, it would appear that the demonstration of multiple tumors in either of these organs in a given patient should be a basis for investigation of hyperfunction of the other, and a search for intrasellar tumor as well. Similarly the coexistence of hyperfunction or tumor in any two of these three endocrine organs should arouse one's suspicion of involvement of the third.

A further point, of practical importance to the surgeon, is the necessity of anticipating the presence of multiple tumors at operation in either the parathyroids or the pancreas in a patient with this syndrome.

SUMMARY

There have been reported in the literature 14 cases in which pituitary tumor, islet-cell adenomas and parathyroid tumors, or a combination of two of these, have occurred in the same patient. Eight additional cases of this syndrome have been encountered at the Mayo Clinic and are reported herein. Parathyroid tumors were present in all, and pituitary tumors were present in 4, of our 8 cases. Five patients had islet-cell tumors and the remaining 3 had hypoglycemia.

Multiplicity of tumors was a striking feature and occurred both in relation to the parathyroids and in relation to the pancreatic islets.

REFERENCES

1. Erdheim, J.: Zur normalen und pathologischen Histologie der Glandula thyreoidea, parathyreoidea und Hypophysis, *Beitr. z. path. Anat. u. z. allg. Path.* **33:** 158–236, 1903.
2. Claude, Henri, and Baudouin, A.: Étude histologique des glandes à sécrétion interne dans un cas d'acromégalie, *Compt. rend. Soc. de biol.* **71:** 75–78, 1911.
3. Josefson, A.: Om endokrina skelett-och utvecklingsrubbningar. Stockholm, Isaac Marcus, 1915, 318 pp. Quoted by Cushing, Harvey and Davidoff, L. M. (4).
4. Cushing, Harvey, and Davidoff, L. M.: The Pathological Findings in Four Autopsied Cases of Acromegaly With a Discussion of Their Significance. (Monograph 22) New York, The Rockefeller Institute for Medical Research, 1927.
5. Lloyd, P. C.: A case of hypophyseal tumor with associated tumor-like enlargement of the parathyroids and islands of Langerhans, *Bull. Johns Hopkins Hosp.* **45:** 1–14 (July) 1929.
6. Hadfield, G., and Rogers, H.: Two parathyroid tumours without osteitis fibrosa: one associated with acromegaly, *J. Path. & Bact.* **35:** 259–263 (Mar.) 1932.
7. Kalbfleisch, H. H.: Adenome inkretorischer Drüsen bei Hypoglykämie, *Frankfurter Ztschr. f. Path.* **50:** 462–477, 1937.
8. Gerstel, Gustav: Über multiple Tumoren der Drüsen mit innerer Sekretion bei einem Akromegalen, *Frankfurter Ztschr. f. Path.* **52:** 485–499, 1938.
9. Shelburne, S. A., and McLaughlin, C. W.: Coincidental adenomas of islet-cells, parathyroid gland and pituitary gland, *J. Clin. Endocrinol.* **5:** 232–234 (May-June) 1945.
10. Rutishauser, E., and Guirguis, Z.: Acromégalie et goitre Langerhansien; syndrome hypophyso-insulaire. *Rev. méd. de la Suisse Rom.* **65:** 585–586, 1945.
11. Burk, L. B., Jr.: Hyperparathyroidism; an analysis of ten cases with special reference to earlier diagnosis, *Am. J. Surg.* n.s. **76:** 404–411 (Oct.) 1948.
12. Crain, E. L., Jr.; Thorn, G. W., and Moore, Francis: Acromegaly with coexisting insulinomas; case report, *Proc. Am. Diabetes A.* **9:** 373–384, 1949.
13. De Ulhôa Cintra, A. B.; Tenuto, Rolando; De Oliveira, H. L., and Flosi, A. Z.: Associação de adenoma da paratireoide com hiperparatireoidismo e adenoma

cromófobo da hipófise—persistência de hipofosfatemia após a cura do hiperparatireoidismo, *Rev. Hosp. clín.* **6**: 67–75 (Apr.) 1951.

14. Wernly, Markus, and Berdjis-Chamsi, C.: Les parathyroïdes humaines. Contribution à l'étude des hyperplasies et des adénomes, *Helvet. med. acta.* **13**(suppl. 19): 1–144, 1946.
15. Black, B. K., and Ackerman, L. V.: Tumors of the parathyroid; a review of twenty-three cases, *Cancer* **3**: 415–444 (May) 1950.
16. Malamud, N., and Grosh, L. C.: Hyperinsulinism and cerebral changes; report of case due to islet cell adenoma of the pancreas, *Arch. Int. Med.* **61**: 579–599 (Apr.) 1938.
17. Rienhoff, W. F., Jr., and Lewis, Dean: Surgical affections of the pancreas met with in the Johns Hopkins Hospital from 1889 to 1932, including a report of a case of an adenoma of the islands of Langerhans, and a case of pancreatolithiasis, *Bull. Johns Hopkins Hosp.* **54**: 386–429 (June) 1934.
18. Friedman, N. D.: Chronic hypoglycemia; report of two cases with islet adenoma and changes in hypophysis, *Arch. Path.* **27**: 994–1010 (June) 1939.
19. Kretschmer, H. L.: Parathyroid adenoma and renal calculi, *J. Urol.* **63**: 947–958 (June) 1950.
20. Chasnoff, Julius; Friedfeld, Louis, and Tunick, A. M.: Hyperparathyroidism in a patient with acromegaly, *Ann. Int. Med.* **16**: 162–175 (Jan.) 1942.
21. Esmarch, O.: États épileptiformes durant des crises hypoglycémiques provoqués par des insulinomes, Acta psychiat. et neurol. **19**: 469–482, 1944.
22. Robinson, F. J.; Power, M. H., and Kepler, E. J.: Two new procedures to assist in the recognition and exclusion of Addison's disease: a preliminary report, *Proc. Staff Meet., Mayo Clin.* **16**: 577–583 (Sept. 10) 1941.
23. Rogers, H. M.; Woolner, L. B.; Johns, Sylvia M., and Sprague, R. G.: Multiple parathyroid adenomas associated with islet-cell tumors of the pancreas; report of two cases, with necropsy findings, *M. Clin. North America* **33**: 1141–1156 (July) 1949.
24. Lopez-Kruger, Rodolfo, and Dockerty, M. B.: Tumors of the islets of Langerhans, *Surg., Gynec. & Obst.* **85**: 495–511 (Oct.) 1947.
25. Norris, E. H.: Collective review; the parathyroid adenoma, a study of 322 cases, *Internat. Abstr. Surg.* **84**: 1–41 (Jan.) 1947.
26. Woolner, L. B.; Keating, F. R., and Black, B. M.: Tumors and hyperplasia of the parathyroids: a review of the pathologic findings in 140 cases, *Cancer* **5**: 1069–1088 (Nov.) 1952.
27. Crain, E. L., Jr., and Thorn, G. W.: Functioning pancreatic islet cell adenomas: a review of the literature and presentation of two new differential tests, *Medicine* **28**: 427–447 (Dec.) 1949.
28. Terbrüggen, August: Anatomische Befunde bei spontaner Hypoglykämie infolge multipler Pankreasinseladenome, *Beitr. z. path. Anat. u. z. allg. Path.* **88**: 37–59, 1931–1932.
29. Frantz, Virginia K.: Adenomatosis of islet cells, with hyperinsulinism, *Ann. Surg.* **119**: 824–844 (June) 1944.
30. Maxeiner, S. R., and Bundy, H. E.: Islet-cell tumors of the pancreas, *Surgery* **18**: 171–177 (Aug.) 1945.

THE CONVERSION OF CORTISONE ACETATE TO OTHER ALPHA-KETOLIC STEROIDS*†

ROBERT B. BURTON, M.D., E. HENRY KEUTMANN, M.D. AND CHRISTINE WATERHOUSE, M.D., with the technical assistance of ELIZABETH A. SCHULER, B.S.

From the Department of Medicine, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

WITH the development of a paper chromatographic method for the isolation and identification of the individual corticosteroids in biologic mixtures (1, 2) it has become possible to detect small changes in the excretion of these compounds in human urine. We have previously reported (3) that the chief α-ketolic corticosteroids obtained from the urine of normal persons by continuous ether extraction at pH 1.0 consisted of cortisone and compound F (15 to 40 gamma of each, daily) and of smaller amounts of more polar material also of the $C_{21}O_5$ type.

The present study concerns the changes in the excretion of corticosteroids produced by the parenteral administration of cortisone acetate. The data indicate that in persons with intact adrenal glands as well as in patients with Addison's disease, cortisone administration produces a marked rise in the excretion of not only cortisone but of compound F, pregnane-3α,17α,21-triol-11,20-dione (tetrahydro-compound E), and a fourth more polar component.

SUBJECTS AND METHODS

Subjects

1. *M.W.* was a 3-year-old male who was admitted on the Pediatric Service with acute leukemia of two months' duration. He exhibited a daily fever with temperatures as high as 40° C. and had marked enlargement of liver, spleen and lymph nodes. The white blood cell count was 169,000 on admission, and there was moderate anemia. He was given cortisone acetate intramuscularly in doses varying from 10 to 50 mg. daily over a three-month period. Although there was a marked lowering of the white cell count and some diminution in the size of the spleen during administration of the higher doses of cortisone, the clinical course was steadily downhill. He died 103 days after admission, while still receiving cortisone. Two pooled urine specimens were collected. One repre-

Received for publication May 5, 1952.

* Parts of these studies were reported at the Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, N.J., June 7–9, 1951.

† This investigation was supported by research grants from the National Cancer Institute of the National Institutes of Health, United States Public Health Service, and from the Upjohn Company, Kalamazoo, Michigan.

sented a 10-day excretion before cortisone therapy, the other a 6-day excretion while receiving 50 mg. of the steroid daily. The latter specimen was collected after the patient had received from 10 to 50 mg. of cortisone daily for thirty days.

2. *J.Ma.*, a well-nourished 30-year-old white male, had had acute rheumatic fever in 1942. He had no further complaints until nine years later, when signs and symptoms of cardiac decompensation developed. He was admitted on the Medical Service five days after the onset of this difficulty. There was no evidence of re-activation of the old rheumatic process. Cardiac compensation was rapidly restored with bed rest, sodium restriction and digitalization. He showed no clinical or laboratory evidence of impaired adrenal function. The present study was undertaken twelve days after admission, at a time when he was entirely symptom-free.

3. *E.L.* was a 45-year-old white female who had been under close supervision and treatment for Addison's disease over a six-year period. She had all the clinical and laboratory findings characteristic of severe adrenal insufficiency, with the usual electrolyte, water, and carbohydrate disturbances. She had been treated with desoxycorticosterone acetate pellets, sodium chloride, testosterone propionate and aqueous adrenal cortex extract (Upjohn). Cortisone therapy had never been employed. For one month before the present study, all hormonal therapy except the desoxycorticosterone acetate pellets had been withdrawn.

4. *J.M.*, a 49-year-old white male, had similarly been under close observation over a five-year period, for Addison's disease. His clinical and laboratory findings were comparable to those in the case of E.L. His therapy had been the same, except that he had received Lipo-adrenal Cortex (Upjohn) in place of the aqueous preparation. For thirty-four days before the present study, all hormonal therapy except the desoxycorticosterone pellets had been discontinued.

5. *B.M.* was a 56-year-old white female who had had lymphosarcoma for five years. Her adrenal glands were considered to be normal. She was studied on the metabolism ward while on a diet roughly comparable to that given E.L. and J.M. Metabolic data obtained on her are included, in order to allow comparison of a normal response to ACTH as witnessed in this clinic, with the responses of E.L. and J.M.

Urinary corticosteroid assays

Urine specimens collected without preservatives were kept at 5°C. until analyzed. The period of storage was never longer than eight days.

Extracts were prepared in two ways. In Method 1, urine specimens were brought to pH 1.0 and continuously extracted with ether for forty-eight hours (3). In certain experiments the urine specimens were divided into two parts. One aliquot was extracted in the above manner; the other was hydrolyzed with β-glucuronidase and then extracted. Extracts were prepared by this second procedure, Method 2, as follows: The urine aliquot was brought to pH 4.5 with 18 N sulfuric acid, and to it was added an amount of 1.0 M acetate buffer (pH 4.5) equivalent to 50 ml. per liter of urine. The buffer contained 57.85 Gm. of sodium acetate ($CH_3COONa \cdot 3H_2O$) and 32.50 ml. of glacial acetic acid per liter of water. A partially purified solution of β-glucuronidase containing 2,000 to 3,000 units per ml. was prepared from fresh beef or calf spleen by the rapid method described for rat tissue (4). Quantities of enzyme representing between 30,000 and 75,000 units per 24-hour sample of urine to be hydrolyzed, were added to the specimens. Incubation was carried out in tightly sealed jars for forty-eight hours at 37°C. The pH was adjusted to 7.0 with 10 per cent sodium hydroxide. Anhydrous sodium sulfate, in amounts

of 70 Gm. per liter of urine, was dissolved in the specimens. The urine was then continuously extracted with U.S.P. ether for forty-eight hours. Extraction was carried out at neutrality rather than at pH 1.0 after enzymatic hydrolysis, since it was found that extraction of most of the pigments, which often complicated the chromatography of the $C_{21}O_4$ and $C_{21}O_3$ urinary corticosteroids (3), could thereby be avoided. Neutral extracts were prepared in the manner outlined previously (3), from the residues obtained by either method.

It is thought that the corticosteroids obtained by Method 1 were present in the urine in the free form, as easily hydrolyzed non-glucuronide conjugates, or both. Extracts prepared by enzymatic hydrolysis, Method 2, were considered to contain corticosteroids originally present in the urine as β-glucuronides, as well as those present in the free form. Although the corticosteroids present as non-glucuronide conjugates which are hydrolyzable at pH 1.0 were probably absent from the latter extracts, it was felt that they constituted only a small fraction of the total excreted. The difference in the corticosteroid content of extracts prepared by the two methods from equivalent aliquots of a urine specimen could be considered, for practical purposes, to correspond to the glucuronide-conjugated corticosteroid.

Neutral extracts of either type were subdivided chromatographically into fractions A, B and C, containing, chiefly, corticosteroids of the $C_{21}O_3$, $C_{21}O_4$ and $C_{21}O_5$ types, respectively. The fractions were then freed of pigments and their individual α-ketolic steroids isolated. The latter were detected with triphenyltetrazolium chloride reagent (TPTZ) (1) and were identified when possible by comparison with crystalline corticosteroids with respect to 1) chromatographic movement of the free compounds as well as of their acetates, 2) special color reactions, 3) ultraviolet absorption curves, and 4) absorption curves of the sulfuric acid chromogens. The methods for these procedures have been described (1, 2, 3, 5).

Quantities of each steroid were estimated by comparing the size and intensity of the spots given by measured aliquots of extract with the spots given by known quantities of pure corticosteroids (2). Results obtained in this way were found to be accurate to within ± 20 per cent of the values obtained by measurement of the optical densities at 240 mμ in the case of compounds having an α, β-unsaturated 3-keto group ($\log_{10}\epsilon = 4.2$; ϵ is expressed as liters $\times$ moles^{-1} $\times$ cm.$^{-1}$).

The use of other micro methods (6, 7, 8) for estimating corticoids quantitatively was felt to offer no advantage in the present study since they measure not only α-ketols but also other steroids which, although not spotted by TPTZ, may have been present on the paper chromatograms. The presence of formaldehydogenic materials in filter paper itself, would have complicated the use of one of these methods (6).

Metabolism studies

Four of the 5 subjects in this report were studied on the metabolism ward of the clinic. In each case, the diet was constant throughout, the

identical menu being used every day. The composition of the daily diets was as follows:

Subject	Calories	Nitrogen (Gm.)	Potassium (mEq.)	Sodium (mEq.)
J.Ma.	2,326	10.22	76.0	12.5
E.L.	2,024	6.70	56.0	105.0
J.M.	2,075	10.17	84.0	147.0
B.M.	2,189	9.50	88.5	84.1

Identical duplicates of the diets as well as urine and stool specimens were analyzed for their content of nitrogen, sodium, and potassium by procedures previously described (9).

Of the urine collected each day, 15 per cent was set aside for the metabolism studies and the remainder, on which the chromatographic analyses for corticosteroids were to be carried out, was put into a pool for each experimental period.

RESULTS

$C_{21}O_5$ *Corticosteroids*

The chief urinary corticosteroids were found in the fraction containing mainly α-ketols of the $C_{21}O_5$ type (Fraction C). Two of these were identified as Kendall's compound F and cortisone, respectively, on the basis of the characteristics previously described (3). A third material found frequently in the urine of normal persons, designated Substance C-1, which chromatographed like a compound more polar than compound F, was also seen in these specimens.

It has been found that substance C-1, on chromatographing in the system, toluene-propylene glycol (methanol-diluted), for 120 hours with a 350-ml. reservoir volume—or in the more rapidly resolving solvent pair, chloroform-formamide (methanol-diluted) (10), for five hours with a 350-ml. reservoir volume—could be subdivided into two distinct materials (Fig. 1).

The more polar of the two, designated Substance C-1a, gave an ultraviolet absorption spectrum which had no maximum. When the material was acetylated, it moved rapidly like a di- or poly-acetate (2). These data indicated the probable presence of a C-3 hydroxyl group. The absorption spectrum of the sulfuric acid chromogen with maxima at 330, 410, and 510 mμ was similar to that given by Reichstein's compound V (maxima at 330, 415, and 510 mμ). The relative heights of the peaks were also similar. It is felt that substance C-1a was either compound V or tetrahydro-compound F, its 3α-hydroxy pregnane isomer, previously

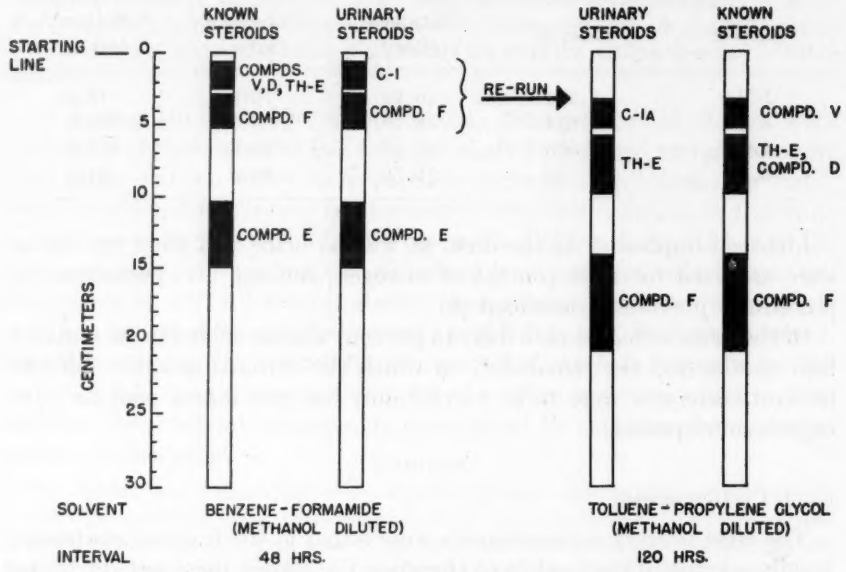


FIG. 1. Diagrams comparing the chromatographic behavior of the principal α-ketolic steroids with crystalline corticosteroids. "Th-E" is tetrahydro-compound E, (pregnane-3α, 17α,21-triol-11,20-dione). Solvent systems and apparatus have been described (1,2). Chromatograms treated with triphenyltetrazolium choride reagent.

isolated from urine by Lieberman and co-workers (11). Although crystalline samples of these two steroids were not available for mixed chromatograms (2) with substance C-1a, the latter moved at a rate very similar to that observed previously with compound V (2), and at a rate one might postulate for the compound described by Lieberman *et al.* This postulation is based upon the similarity of movement of compound D (Reichstein) and tetrahydro-compound E, an analogous pair of isomers. We believe it more likely that substance C-1a is the pregnane, tetrahydro-compound F, since no α-ketolic allopregnanes, to which class compounds D and V belong, have been found in the urine.

Substance C-1b, the faster moving of the two components had no absorption maximum in the ultraviolet region of the spectrum. The sulfuric acid chromogen curve of the acetate had peaks at 340 and 412 mμ and was similar in shape to the curve obtained by Zaffaroni (12) with a crystalline sample of pregnane-3α, 17α,21-triol-11,20-dione 3,21-diacetate. In a mixed chromatogram with the latter, acetylated substance C-1b gave a single

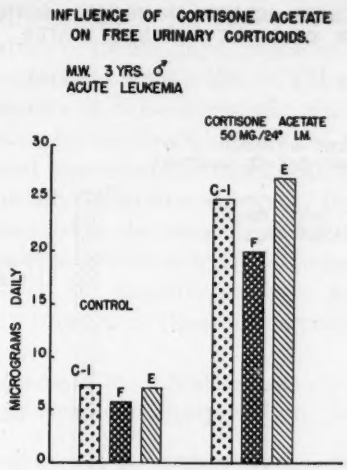


FIG. 2. Changes in urinary excretion of the $C_{21}O_5$ α-ketolic steroids in M.W., the 3-year-old boy with acute leukemia, upon intramuscular administration of cortisone acetate. Urine extracted at pH 1.0 (Method 1, *see* text). C-1 represents substance C-1; F represents Kendall's compound F, 17-hydroxycorticosterone; and E represents cortisone.

spot. The solvent system used was cyclohexane-formamide (methanol-diluted); the development time was forty hours with 350 ml. of cyclohexane in the reservoir trough. These data strongly suggest that substance C-1b is pregnane-3α, 17α,21-triol-11,20-dione,tetrahydro-compound E, which has been isolated from urine and characterized by Lieberman *et al.* (13) and by Schneider (14).

In Figure 2 are presented the changes in the excretion of steroids in the $C_{21}O_5$ fraction, produced by the daily intramuscular administration of 50 mg. of cortisone acetate to the child, M.W. Urine specimens in this case were extracted only at pH 1.0 (Method 1). Because of the small quantities excreted, no attempt was made to subdivide substance C-1 by prolonged chromatographing. Initially, he excreted daily between 5 and 10 gamma each of compound F, cortisone, and substance C-1. During cortisone therapy, the daily excretion of all constituents rose to 20 to 30 gamma of each.

In the case of J. Ma. (Fig. 3), urinary corticoids were extracted by each of the two methods described. During cortisone administration, increased excretion of all components was seen. The greater the dose of cortisone, furthermore, the greater was the increase observed. Substance C-1a and tetrahydro-compound E each showed a greater rise than did either cortisone or compound F. Most of the increased excretion of the first two compounds, but not of the latter two, was attributable to glucuronide-conjugated steroids.

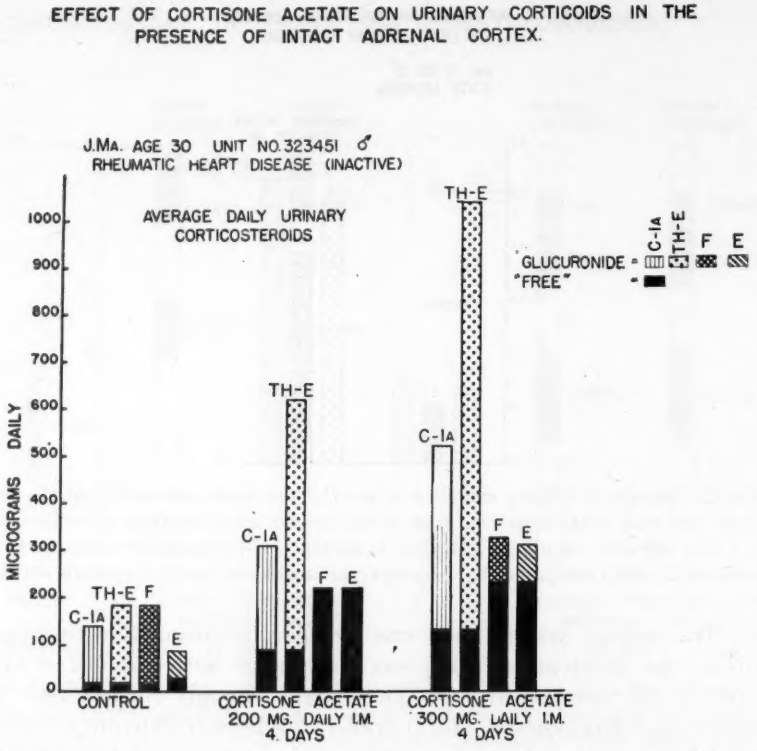


FIG. 3. Response of urinary α-ketols of the $C_{21}O_5$ type in J. Ma., a male with normal adrenocortical function, upon administration of cortisone acetate intramuscularly. Solid areas of the bars indicate the amounts of each steroid present in the urinary aliquot extracted by Method 1. The total height of each bar represents the amount of steroid present in the aliquot of urine treated with β-glucuronidase and extracted by Method 2.

These findings indicated the probability that cortisone was converted to substance C-1a and tetrahydro-compound E and compound F, but raised the question of whether the transformation occurred in the patient's adrenal cortices or whether some other site was responsible.

The following studies, therefore, were carried out on the 2 patients with Addison's disease (E.L. and J.M.). After a period for control observations, the patients were given 100 mg. daily of adrenocorticotropic hormone intramuscularly for eight days (E.L.) or four days (J.M.), in an effort to demonstrate the absence of significant adrenocortical function, both metabolically and from the viewpoint of corticosteroid excretion. After further control studies, cortisone acetate in doses of 100 to 200 mg. daily

was administered intramuscularly for twelve or eight days, followed by control periods of sixteen or twelve days, respectively. In the case of E.L., a period of six weeks elapsed between the ACTH and cortisone regimens

In Table 1, the results of these metabolic studies are recorded. The ACTH was of the same lot number (Armour # J 17505) in all instances. It produced the typical response in nitrogen balance in the case of B.M., the subject with normally responsive adrenals. In both Addisonians, no significant change occurred in this respect during the treatment.

The commonly observed effects of ACTH administration on electrolyte excretion occurred in B.M., namely, negative potassium and positive sodium balances, with reversal of these phenomena on withdrawal of the hormone.

The electrolyte balances of the 2 Addisonians when they received ACTH are less susceptible to clear cut interpretation. Neither patient showed

TABLE 1. METABOLIC CHANGES WITH ADMINISTRATION OF ACTH AND CORTISONE ACETATE INTRAMUSCULARLY

Patient	Diagnosis	Treatment	Duration (days)	Balances*			Treatment	Duration (days)	Balances*		
				Nitrogen (Gm./24 hrs.)	Potassium (mEq./24 hrs.)	Sodium (mEq./24 hrs.)			Nitrogen (Gm./24 hrs.)	Potassium (mEq./24 hrs.)	Sodium (mEq./24 hrs.)
E.L.	Addison's disease	ACTH, 100 mg./day	8	+0.2	+ 2.0	−16	Cortisone, 100 mg./day	8	−1.3	− 1.2	−29
		Post-ACTH control	8	+0.7	+ 3.0	+11	Cortisone, 200 mg./day	4	−2.1	− 0.2	−11
							Post-cortisone control	6	−2.7	+ 4.9	−15
								10	−0.9	− 1.0	−18
J.M.	Addison's disease	ACTH, 100 mg./day	4	−0.1	− 2.0	+ 7.0	Cortisone, 100 mg./day	4	−0.3	− 3.0	−13
		Post-ACTH control	6	−0.3	− 8.0	+15	Cortisone, 100 mg./day	4	−1.2	+12	−28
							Post-cortisone control	12	−0.7	+ 8.0	−33
B.M.	Lympho-sarcoma	ACTH, 100 mg./day	4	−2.4	−36	+82					
		Post-ACTH control	4	−1.7	+15	−134					
J.Ma.	Rheumatic heart disease (inactive)						Cortisone, 200 mg./day	4	−2.1	− 2.0	+ 8.0
							Cortisone, 300 mg./day	4	−5.0	0.0	+12
							Post-cortisone control	4	−5.9	+ 5.0	+15

* Balances are calculated as the average daily changes from the immediately preceding pre-treatment control periods.

significant loss of potassium during treatment nor the expected retention when the hormone was withdrawn.

Although changes that may have been significant occurred in the sodium balances of these 2 patients, they were not those usually seen with stimulation of the adrenal cortex by ACTH. It is possible that they resulted from the posterior pituitary principle present in the preparation. The variable effect on the excretion of electrolytes which has been produced when this antidiuretic hormone was administered to human subjects under differing experimental conditions has been discussed recently by several authors (15, 16).

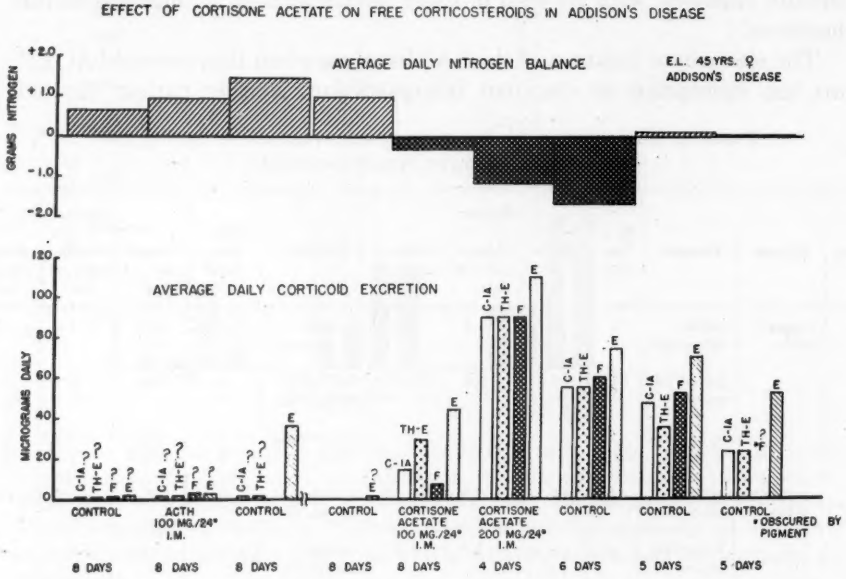


FIG. 4. Changes in the urinary excretion of corticosteroids of the $C_{21}O_5$ type and in nitrogen balance in the female Addisonian, E.L., during ACTH and cortisone administration. Urine extracted for corticosteroids by Method 1. A period of six weeks elapsed between the post-ACTH and pre-cortisone control periods.

During cortisone administration, the subjects with Addison's disease, like the normal subject, J.Ma., showed a negative nitrogen balance. The changes in sodium and potassium balances during the therapy were similar to those observed in such patients by Thorn and Forsham (17) and by Perera *et al.* (18). As pointed out by the former authors, these results could be produced by two compounds, desoxycorticosterone and cortisone, whose actions on the renal tubules are qualitatively similar but quantitatively unequal and simultaneously competitive.

Urinary corticosteroid assays in the case of E.L. were carried out only by Method 1. The results of these and the nitrogen balances are presented in Figure 4. Only trace amounts of α-ketolic material were found during the control period. The amounts were too small for identification but the positions of the spots on the chromatograms corresponded to those of C-1a, tetrahydro-compound E, compound F, and cortisone. During therapy with 100 mg. daily of ACTH intramuscularly for eight days, no significant change was seen in corticosteroid excretion.

The excretion of approximately 35 gamma of cortisone daily during the first control period after ACTH is difficult to explain; we do not feel that it was due to activation of adrenocortical remnants. When ACTH has been given to patients with normally responsive adrenal glands, a more prompt rise in corticosteroid excretion has been seen and, furthermore, the output of compound F has always been greater than that of cortisone (19). We believe that the anomalous finding of an increase of only cortisone in this specimen was probably due to its inadvertent contamination in the laboratory by crystalline cortisone, a steroid most frequently used as reference material on paper chromatograms.

Upon receiving 100 mg. of cortisone daily for eight days and then 200 mg. daily for four days, there was a prompt increase of all four of the corticosteroids described above. Average values of about 100 gamma daily of each steroid were reached during the period on the higher dosage. Following cortisone administration, increased steroid excretion continued for the sixteen days the patient was observed. The improvement in appetite, strength, and mental outlook which took place persisted for five months, although no more of the steroid was given.

In the case of J.M., the second Addisonian, urinary corticosteroid analyses were performed by each of the two methods described. The urinary corticosteroid excretion and the nitrogen balances are shown in Figure 5. There was no free corticosteroid during the control period and less than 20 gamma per twenty-four hours each of C-1a, tetrahydro-compound E, compound F, and cortisone in the form of glucuronide. As in the case of E.L., the amounts at this time were too small for identification but the chromatographic positions indicated that these were the probable identities.

No response in corticoid excretion was seen during therapy with 100 mg. daily of ACTH intramuscularly for four days. During the first four days of treatment with 100 mg. of cortisone acetate daily, there was a very sharp rise in the excretion of tetrahydro-compound E glucuronide, a moderate increase in C-1a glucuronide and cortisone glucuronide, and a small increase in compound F glucuronide. Small amounts of these compounds appeared for the first time in the aliquot of urine extracted at pH

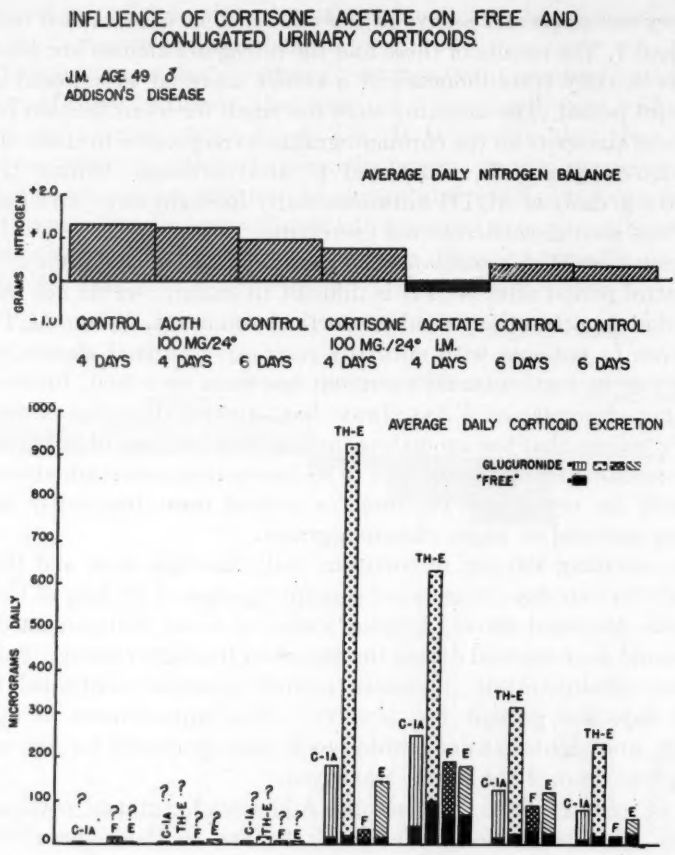


FIG. 5. Excretion of urinary corticosteroids of the $C_{21}O_5$ type and nitrogen balances in J.M., a male with Addison's disease, during ACTH and cortisone administration. Solid areas of the columns indicate α-ketols extractable from the aliquot of urine at pH 1.0 (Method 1). Total height of each column represents the amount of the particular steroid found in the aliquot of urine extracted by Method 2.

1.0. During the second period of therapy with 100 mg. of intramuscular cortisone, the total excretion of compound F continued to rise, while that of tetrahydro-E fell somewhat. The excretion of C-1a and cortisone changed only slightly. In all cases, the excretion of steroid extractable at pH 1.0 was higher than it had been during the first cortisone period. Following omission of cortisone, the excretion of all components gradually fell; but twelve days after the last dose, the urinary corticosteroids continued to be higher than during the pre-cortisone periods.

Corticosteroids of the $C_{21}O_4$ *and* $C_{21}O_3$ *types*

In a previous study (3) of the excretion by normal persons of corticosteroids extractable from urine at pH 1.0 (Method 1), it was found that only occasionally were α-ketolic materials found in the fractions of the extracts in which most of the $C_{21}O_4$ steroids might be found. No α-ketols were found in the $C_{21}O_3$ fractions. For these reasons, analyses of these fractions of extracts were carried out only on selected specimens in the present study.

$C_{21}O_4$ *Corticosteroids*

During the control period, M.W. excreted only traces of α-ketolic material (pH 1.0 extractable) which, in the chromatograms, moved at about the rate of corticosterone. The small amounts precluded further attempts at identification. In an extract representing a 10-day specimen of urine, a total of less than 10 gamma was found. While the patient was receiving 50 mg. of intramuscular cortisone acetate daily, there was no significant change in the amount in a pooled specimen representing a 6-day excretion.

The control specimens from J.Ma. contained three unidentified α-ketolic substances in the fractions which should have contained chiefly $C_{21}O_4$ steroids. Amounts up to 40 gamma per day of each were excreted in the form extractable by Method 1 and somewhat larger amounts, up to 250 gamma per day, as β-glucuronides. No significant change was noted when 200 mg. of cortisone acetate daily was given.

J.M. excreted, during the control period, only traces of three unidentified α-ketols which moved like $C_{21}O_4$ corticosteroids in extracts prepared by Method 1, and between 20 and 80 gamma daily of each in the extracts prepared by Method 2. During administration of 100 mg. of cortisone daily, no change was seen.

$C_{21}O_3$ *Corticosteroids*

Subject M.W. did not excrete detectable amounts of 11-desoxycorticosterone during either the control or the cortisone periods referred to previously. In this case, the $C_{21}O_3$ fraction of the urinary extract was analyzed only for 11-desoxycorticosterone in the form of the etiocholenic acid derivative (3).

J. Ma., during the control period, excreted approximately 20 gamma per day of an unidentified, pH 1.0-extractable, α-ketol which moved like a steroid of the $C_{21}O_3$ type. While he received 200 mg. of cortisone acetate daily, no increase was found in the aliquot extracted by Method 2. The aliquot of the same urine extracted by Method 1, however, showed approximately 180 gamma per day of this material. The increment may repre-

sent material excreted neither in the free form nor as glucuronide, but as a conjugate hydrolyzable at pH 1.0.

J.M. excreted no pH 1.0-extractable steroids of the $C_{21}O_3$ type before cortisone therapy. About 10 gamma per day of an unidentified α-ketol was found in the glucuronidase-treated specimens. During the two periods of treatment with 100 mg. of cortisone acetate, there was no change in the excretion of "free" steroid, but the glucuronide-conjugated material increased to about 50 gamma per day.

DISCUSSION

Several explanations might be offered for the increased excretion of compound F, tetrahydro-compound E, and tetrahydro-compound F in the 2 patients with intact adrenal glands, M.W. and J.Ma., during cortisone administration. It is conceivable that the three excreted steroids had been produced previously by the adrenal cortices of the subjects and that the administered cortisone resulted in the displacement and excretion of those compounds. It may also be postulated that the cortisone was transformed by the intact adrenals.

These possibilities obviously do not apply in the subjects with Addison's disease. It appears, rather, that the cortisone was converted in non-adrenal tissues to the other three compounds. Figure 6 indicates that one or more relatively simple changes in structure are necessary to convert cortisone to these other three steroids, namely, reduction of the C-3 and C-11 keto groups and of the unsaturated bond in Ring A.

The extent to which injected cortisone acetate was converted to the other three $C_{21}O_5$ steroids and then excreted in a form rendering them ether-extractable from urine at pH 1.0, was comparable in the 2 Addisonians to that in the subject with normal adrenals, J.Ma. The rate of conversion to α-ketols excreted as β-glucuronides, furthermore, was of the same order of magnitude in Subject J.M. (the only Addisonian in whom glucuronides were determined) as in Subject J.Ma. These observations suggest that the extra-adrenal mechanism was the most important one, even in the patients with normal glands. The precise site at which these changes occurred is not indicated by the present studies.

It is of interest that cortisone administration caused no increase in excretion of steroids of the $C_{21}O_4$ type. On the other hand, in 1 Addisonian (J.M.) and in 1 of the 2 subjects with normal adrenals (J.Ma.) in whom $C_{21}O_3$ steroids were studied, a significant increase in excretion of these steroids was seen. These data are insufficient for final conclusions in regard to the body's capability for removing oxygen atoms from the steroid molecule.

The increment in the excretion of α-ketols (free plus glucuronides) at

POSSIBLE STEPS IN THE METABOLISM OF CORTISONE.

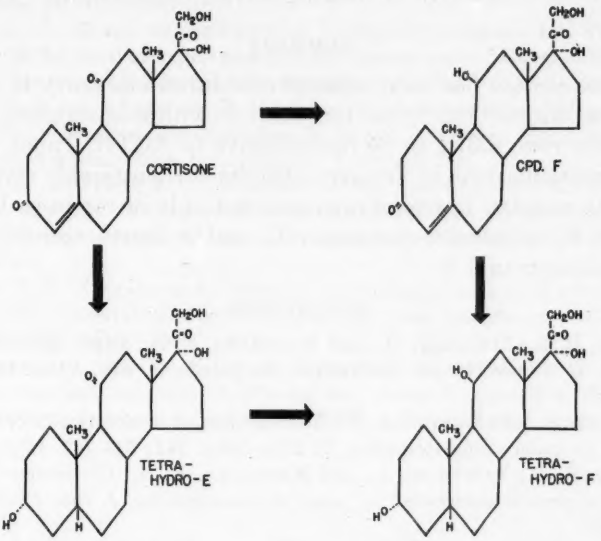


FIG. 6. Structural formulae of cortisone, compound F, tetrahydro-compound E, and tetrahydro-compound F, showing the relatively simple changes necessary for conversion of cortisone to the latter three steroids.

the time of cortisone administration in J.Ma., and in the Addisonian, J.M., accounted for approximately 0.3 per cent and 1.0 per cent, respectively, of the amount being given. The total excreted by J.M. during the eight days of cortisone therapy and during the twelve days following, accounted for approximately 2.0 per cent of the steroid given. The eventual amount to be recovered was no doubt somewhat higher. This is suggested by the data in E.L., which show that increased excretion of free and pH 1.0-hydrolyzable steroids persisted for at least sixteen post-treatment days and, if her clinical improvement could be taken as a guide, may have continued for several months. This relatively long period of excretion may indicate either that the drug was liberated from the injection site at a relatively slow rate or that the excretion of steroid, once it entered the body fluids, was slow.

The demonstration that cortisone is convertible to compound F in the body raises the possibility that the physiologic effects of injected cortisone may actually be due to the compound F. In this regard, it is interesting

that the observations of Hollander *et al.* (20) indicate that the antirheumatic effect of cortisone, but not of compound F, is absent when the steroid is injected into the joint space. Tissues may vary widely in their ability to convert cortisone to compound F; for example, locally administered cortisone is effective in treating certain inflammatory eye diseases.

SUMMARY

Cortisone acetate has been administered intramuscularly to 2 subjects with normal adrenal glands and to 2 subjects with long standing Addison's disease, who were shown to be unresponsive to ACTH. Paper chromatographic determinations of urinary α-ketolic corticosteroids revealed that all subjects excreted increased amounts, not only of cortisone but also of compound F, tetrahydro-compound E, and a fourth steroid, probably tetrahydro-compound F.

REFERENCES

1. Burton, R. B.; Zaffaroni, A., and Keutmann, E. H.: Paper chromatography of steroids. II. Corticosteroids and related compounds, *J. Biol. Chem.* **188:** 763–771, 1951.
2. Zaffaroni, A., and Burton, R. B.: Identification of corticosteroids of beef adrenal extract by paper chromatography, *J. Biol. Chem.* **193:** 749–767, 1951.
3. Burton, R. B.; Zaffaroni, A., and Keutmann, E. H.: Corticosteroids in urine of normal persons determined by paper chromatography, *J. Biol. Chem.* **193:** 769–779, 1951.
4. Talalay, P.; Fishman, W. H., and Huggins, C.: Chromogenic substrates. II. Phenolphthalein glucuronic acid as a substrate for the assay of glucuronidase activity, *J. Biol. Chem.* **166:** 757–772, 1946.
5. Zaffaroni, A.: Absorption spectra of sulfuric acid chromogens obtained from adrenal steroids and related compounds, *J. Am. Chem. Soc.* **72:** 3228, 1950.
6. Daughaday, W. H.; Jaffe, H., and Williams, R. H.: Chemical assay for "cortin." Determination of formaldehyde liberated on oxidation with periodic acid, *J. Clin. Endocrinol.* **8:** 166–174, 1948.
7. Talbot, N. B.; Saltzman, A. H.; Wixom, R. L., and Wolfe, J. K.: The colorimetric assay of urinary corticosteroid-like substances, *J. Biol. Chem.* **160:** 535–546, 1945.
8. Heard, R. D. H., and Sobel, H.: Steroids. VIII. A colorimetric method for the estimation of reducing steroids, *J. Biol. Chem.* **165:** 687–698, 1946.
9. Waterhouse, C.; Bassett, S. H., and Holler, J. W.: Metabolic studies on protein depleted patients receiving a large part of their nitrogen intake from human serum albumin administered intravenously, *J. Clin. Investigation* **28:** 245–264, 1949.
10. Zaffaroni, A., and Burton, R. B.: Unpublished observations.
11. Lieberman, S.; Hariton, L. B.; Stokem, M. B.; Studer, P. E., and Dobriner, K.: Steroid excretion after administration of ACTH in man, *Fed. Proc.* **10:** 216, 1951.
12. Zaffaroni, A.: Personal communication.
13. Lieberman, S.; Hariton, L. B., and Dobriner, K.: Alterations in steroid excretion after administration of ACTH in man, *Fed. Proc.* **9:** 196, 1950.

14. SCHNEIDER, J. J.: Studies on the excretion of adrenocortical compounds. I. Isolation of 17-hydroxy-11-dehydrocorticosterone and other compounds from the urine of normal males, *J. Biol. Chem.* **183:** 365–376, 1950.
15. MURPHY, R. J. F., and STEAD, E. A., JR.: Effects of exogenous and endogenous posterior pituitary antidiuretic hormone on water and electrolyte excretion, *J. Clin. Investigation* **30:** 1055–1061, 1951.
16. WHITE, A. G.; RUBIN, G., and LEITER, L.: Studies in edema. III. The effect of pitressin on the renal excretion of water and electrolytes in patients with and without liver disease, *J. Clin. Investigation* **30:** 1287–1297, 1951.
17. THORN, G. W., and FORSHAM, P. H.: Metabolic changes in man following adrenal and pituitary hormone administration, *in* Recent Progress in Hormone Research. New York, Academic Press, Inc., 1949, vol. 4, pp. 229–288.
18. PERERA, G. A.; PINES, K. L.; HAMILTON, H. B., and VISLOCKY, K.: Clinical and metabolic study of 11-dehydro-17-hydroxy-corticosterone acetate (Kendall compound E) in hypertension, Addison's disease and diabetes mellitus, *Am. J. Med.* **7:** 56–69, 1949.
19. BURTON, R. B.; ZAFFARONI, A.; IZZO, A. J., and KEUTMANN, E. H.: Studies on the nature of the adrenal cortical secretion in patients with malignancy, *J. Clin. Investigation* **30:** 632, 1951.
20. HOLLANDER, J. L.; BROWN, E. M., JR.; JESSAR, R. A., and BROWN, C. Y.: Hydrocortisone and cortisone injected into arthritic joints. Comparative effects of and use of hydrocortisone as a local anti-arthritic agent, *J.A.M.A.* **147:** 1629–1635, 1951.

ANTIDIURETIC ACTIVITY OF THE SERUM OF NORMAL AND DISEASED SUBJECTS*

W. F. PERRY, M.D. AND T. W. FYLES, M.D.

From the Department of Physiology and Medical Research, The University of Manitoba and from the Winnipeg General Hospital, Winnipeg, Canada

INTRODUCTION

IT IS well known that patients with congestive heart failure excrete water less readily than do normal humans. Edema is liable to develop, and it is possible that this is due to the action of an antidiuretic principle. Thus Robinson and Farr (1) in 1940, investigating certain edematous states of noncardiac origin, found increased amounts of an antidiuretic substance in the urine; and Bercu *et al.* and others (2, 3) recently reported similar findings in subjects with congestive heart failure.

Eversole, Birnie and Gaunt (4, 5) have shown in rats that the liver inactivates Pitressin. From this observation one might expect that patients with liver damage would have increased amounts of antidiuretic substances in the body, and in fact, Ralli *et al.* (6) have found that cirrhotic patients with ascites excreted more than normal amounts of an antidiuretic substance. Hall, Frame and Drill (7) also have reported that the urine of cirrhotics contains a substance with antidiuretic activity, but they were unable to demonstrate such activity in the urine of normal subjects. Recently Dochios and Dreifus (3) have shown an increased antidiuretic activity in the urine of patients with cirrhosis.

Accordingly, it appeared of interest to determine the levels of antidiuretic activity in the serum of patients with cardiac edema and in the serum of patients with liver disease, using the method devised by Birnie *et al.* (8, 9).

METHODS

The assay of serum antidiuretic substance was made on venous blood. The serum was separated from the cells as rapidly as possible by centrifugation and used for assay within one hour of being drawn. This time limit was imposed because, in agreement with Birnie (8), the activity was found to be markedly diminished after twenty-four hours of storage. The assay was performed on male rats, each weighing approximately 200 Gm. The Heller and Urban procedure, as modified by Birnie *et al.* (8, 9),

Received for publication May 19, 1952.

* The material of this paper is part of a thesis submitted to the Faculty of Graduate Studies, The University of Manitoba, by Dr. Fyles in partial fulfillment for the degree of Master of Science.

was used with further slight modifications. The rats were isolated in a quiet room, fasted, but allowed water without restriction for eighteen hours before being used. Test groups of 3 rats were placed in metabolism cages. Tap water, in an amount corresponding to 5 per cent of the body weight and warmed to body temperature, was administered to the animals by gavage. This dose was repeated one hour later, and one hour after this second dose the urine from each group was measured, then discarded. A third similar dose of water was then administered and the test material (serum, or physiologic saline) injected intraperitoneally. The dose of each test material was 0.5 ml. per 100 Gm. of rat. Following this, the urine output of each group was measured after three hours and in many cases the chloride content was determined by the Volhard-Harvey method (10).

The water excreted during the three hours was expressed as its percentage of the "water load," *i.e.*, the total amount of water given, minus that amount excreted prior to injection of the test material. No animals were used at intervals of less than seven days and those which received serum were not used more than twice, because of the possibility of protein sensitization.

In the various control saline-injected groups it was found that the percentage of the "water load" which was excreted by the end of the experimental period varied from 64 to 79 per cent with a mean of 72.6 per cent. In all but a few cases the experimental serum-injected groups excreted less water than did their controls. Antidiuretic activity was expressed in units (A.D. units); these were calculated by subtracting the percentage of the "water load" excreted by the serum-injected rats from the percentage of the "water load" excreted by the control saline-injected rats, in experiments run simultaneously.

RESULTS

The variability of the assay procedure is shown in Table 1 by the values obtained on the three separate groups of animals injected simultaneously with serum from a subject with heart failure. These three groups excreted 36.8, 33.8, and 37.7 per cent, respectively, of the "water load," giving antidiuretic values for this particular serum of +35.6, +38.6, and +34.7 A.D. units. Similar determinations were made, using the serum from a

TABLE 1. THE VARIABILITY OF THE ASSAY PROCEDURE

Assays	Per cent "water load" excreted			Antidiuretic activity (A.D. units)		
	Cage 1	Cage 2	Cage 3	Cage 1	Cage 2	Cage 3
Serum I (Normal)	73.1	72.7	71.8	+ 2.4	+ 2.8	+ 3.7
Control	75.5					
Serum II (Heart failure)	36.8	33.8	37.7	+35.6	+38.6	+34.7
Control	72.4					

normal subject with very little antidiuretic activity. Figure 1 shows serum levels of antidiuretic activity in different degrees of tissue hydration. Two normal males ingested 6.5 liters of tapwater over a four-hour period. At the end of this time their sera were found to be slightly diuretic, the antidiuretic activity being −2 A.D. units in each case. One week later the same 2 subjects withheld all fluids for thirty hours, solid food only being consumed during this time. The antidiuretic activity of the serum at the end of this period was found to be +12 and +23 A.D. units, respectively. A somewhat similar difference was found between the serum of a rat allowed to drink water without restriction and that of a rat in which water was withheld for eighteen hours.

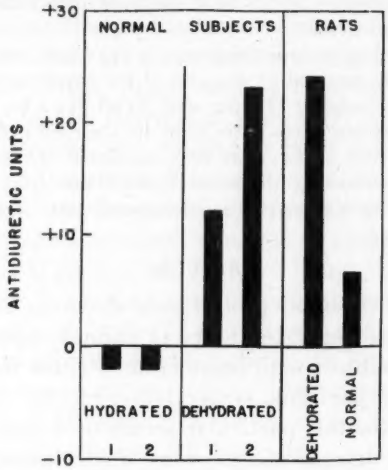


FIG. 1. The effect of hydration on the serum antidiuretic activity.

In Figure 2 are shown the results of 16 determinations of antidiuretic activity in the serum of 13 normal subjects in a normal state of hydration. In Cases 2, 6, and 8 determinations were made on two different occasions. The activity ranged from −7 to +34.5 A.D. units with an average of +16.6 units. In Table 2 are shown the results of 12 separate estimations on 10 subjects with congestive heart failure. All these patients had gross pitting edema and ascites; all but 4 had pleural effusions. They were admitted to the hospital because of symptoms associated with increasing water retention. The sera were tested before treatment was instituted and as soon as possible after admission. The range of antidiuretic activity was from +2.5 to +42.1 A.D. units, with an average of +23.2 units—higher than the mean results for normal subjects. However, when the results in the two groups were compared by an analysis of variance it

was found that the difference was not significant ($P > 0.05$). The level of antidiuretic activity in the group of cardiac patients did not correspond to the extent of edema; data for fluid intake were not available for correlation.

Table 3 shows the extent of liver damage in a group of 9 patients with liver disease. All patients had an increased level of serum bilirubin and abnormal results from cephalin flocculation tests; all but 1 had abnormal results from thymol turbidity tests. Twelve separate estimations of anti-

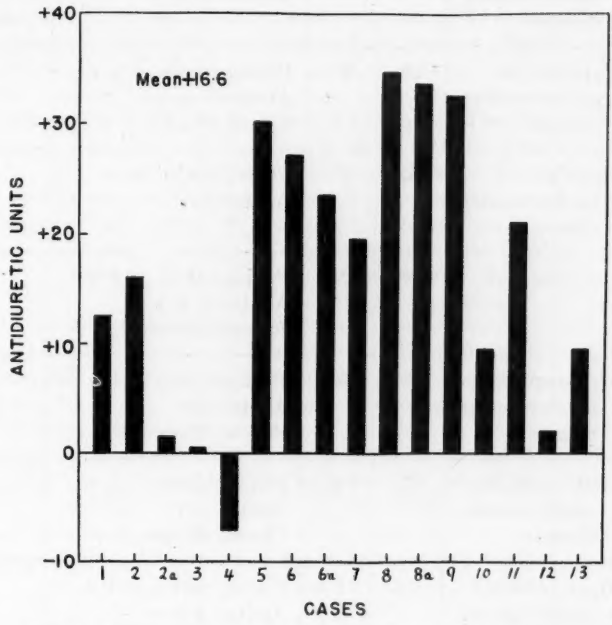


FIG. 2. Serum antidiuretic activity of normal subjects

diuretic activity were carried out on the 9 patients. The range of antidiuretic activity was from +4.5 to +37 A.D. units, with an average of +20.9 units. An analysis of variance showed that there was no significant difference between this group and the normal group ($P > 0.05$). The antidiuretic activity of the patients' sera could not be correlated with the extent of liver damage.

The sera from 3 cases of Addison's disease and from single cases of the nephrotic syndrome, lymphatic edema, Hodgkin's disease and lymphatic leukemia were tested. As shown in Table 4 the antidiuretic values fell within the normal range.

TABLE 2. DETAILS OF SUBJECTS WITH CONGESTIVE HEART FAILURE

Case No.	Diagnosis	Age (yrs.)	Sex	Clinical summary	Antidiuretic activity (A.D. units)
1	Rheumatic heart disease	49	F	Pitting edema +++ Ascites ++	+33 + 5.5
2	Hypertensive cardiovascular disease	77	M	Pitting edema ++ Ascites ++	+28.5 +24.5
3	Hypertensive cardiovascular disease	69	F	Pitting edema +++ Ascites +++ Pleural effusion ++	+27
4	Hypertensive cardiovascular disease	51	F	Pitting edema ++ Ascites ++	+37
5	Cor pulmonale	68	M	Pitting edema ++++ Ascites +++ Pleural effusion +++	+42.1
6	Hypertensive cardiovascular disease	77	M	Pitting edema +++ Ascites ++ Pleural effusion ++	+39.5
7	Arteriosclerotic cardiovascular disease	77	M	Pitting edema +++ Ascites ++ Pleural effusion ++	+ 2.5
8	Hypertensive cardiovascular disease	48	F	Pitting edema +++ Ascites +++ Pleural effusion ++	+24.5
9	Cor pulmonale	58	M	Pitting edema ++++ Ascites ++	+ 2.5
10	Arteriosclerotic cardiovascular disease	69	F	Pitting edema +++ Ascites ++ Pleural effusion ++	+12
					Mean+23.2

TABLE 3. DETAILS OF SUBJECTS WITH LIVER DISEASE

Case No.	Diagnosis	Age (yrs.)	Sex	Clinical summary	Antidiuretic activity (A.D. units)
1	Portal cirrhosis	63	F	Gross ascites and edema. Serum bilirubin 4.5 mg.%. Ceph. flocc. +++. Thymol turb. 0.04.	+22.5 + 7.5
2	Infectious hepatitis	24	M	Serum bilirubin 11.9 mg.%. Ceph. flocc. ++++. Thymol turb. 0.04.	+36.5 +25.1
3	Obstructive jaundice	74	M	Serum bilirubin 19.2 mg.%. Ceph. flocc. +++. Thymol turb. 0.05.	+35.1
4	Homologous serum jaundice	40	F	Serum bilirubin 4.5 mg.%. Ceph. flocc. ++++. Thymol turb. 0.09. Prothrombin time 48%.	+ 5.1
5	Leukemic infiltration of the liver	72	F	Serum bilirubin 16.0 mg.%. Ceph. flocc. ++++. Thymol turb. 0.3.	+ 4.5
6	Obstructive jaundice	69	M	Serum bilirubin 13.5 mg.%. Ceph. flocc. +++. Thymol turb. 0.04.	+26.1
7	Portal cirrhosis	80	M	Serum bilirubin 14.3 mg.%. Ceph. flocc. ++. Thymol turb. 0.01.	+ 7.0 +37.0
8	Infectious hepatitis	28	M	Serum bilirubin 8.2 mg.%. Ceph. flocc. +++. Thymol turb. 0.07.	+14.5
9	Obstructive jaundice	67	M	Serum bilirubin 2.5 mg.%. Ceph. flocc. +++. Thymol turb. 0.08.	+30.0
					Mean+20.9

TABLE 4. SERUM ANTIDIURETIC ACTIVITY

Case No.	Diagnosis	Age (yrs.)	Sex	Antidiuretic activity (A.D. units)
1	Addison's disease	54	F	+18.5
2	Addison's disease	21	M	+20.0
3	Addison's disease	32	M	+14.5
4	Nephrotic syndrome	62	M	+21.0
5	Lymphatic edema	60	M	+16.5
6	Hodgkin's disease	20	M	+37.6
7	Lymphatic leukemia	50	M	− 3.5

The chloruretic activity of the sera was estimated by comparing the amount of chloride excreted in the three hours after the injection of the test material with the amount excreted by the animals injected with saline. As shown in Table 5 human serum had no effect on chloride excretion, despite an effect on water excretion. It thus differed from Pitressin, which was chloruretic as well as antidiuretic.

TABLE 5. COMPARISON OF CHLORURETIC AND ANTIDIURETIC ACTIVITY

Substance	Chloride excretion (mg. in 180 mins.)			Antidiuretic activity (A.D. units)		
	No. of estimations	Average	Range	No. of estimations	Average	Range
Saline	11	12.8	6.5– 20.4	11	0	—
Pitressin, 5 milliunits	9	46.5	28.0– 67.0	9	12.6	2.0–29.6
Pitressin, 10 milliunits	8	60.8	29.7– 91.0	8	18.3	10.0.27.5
Pitressin, 20 milliunits	9	76.5	34.0–113.0	10	31.6	22.5–48.0
Sera—Normal subjects	12	11.4	5.4– 22.2	16	16.6	−7.0–34.5
Sera—Congestive heart failure	11	8.9	0.0– 22.2	12	23.2	2.5–42.1
Sera—Liver disease	9	10.9	3.0– 31.7	12	20.9	4.5–37.0
Sera—Miscellaneous	5	8.4	3.0– 13.8	7	17.4	−3.5–35.0

DISCUSSION

Birnie *et al.* (8, 9, 11) found that rat serum contains a substance with both antidiuretic and chloruretic activity, which is related to the degree of hydration of the animal; for these reasons it is believed by them to originate in the neurohypophysis. Dicker and Ginsberg (12) have also recently shown that the serum of rats contains an antidiuretic substance but they further found that it was not present in plasma. It is not apparent

from their data whether the serum activity is related to hydration or not. They suggest that the active substance is a product of blood coagulation. More recently Gaunt (13) has been unable to confirm the work of Dicker and Ginsberg, finding plasma and serum to be equally antidiuretic. The results of Birnie *et al.* (8, 9, 11) and those reported here for the human, indicate that the amount of this substance can change with the degree of hydration of the body, and for this reason it would seem unlikely that it is an artefact. In addition, several investigators (1, 2, 3, 6, 7) have reported the presence of a substance with similar activity in the urine.

The antidiuretic substance in human serum differs from that in rat serum and also from Pitressin, in not being chloruretic. Whether the substance measured in human serum is derived from the neurohypophysis and whether it is identical with antidiuretic substance excreted in urine, remains to be established. Although the activity in serum from 2 healthy persons has been shown to change with variations in the degree of tissue hydration, judging from the rather wide variations in the values obtained on normal persons taking fluids as desired, it would seem not improbable that the antidiuretic activity measured is a net effect of antidiuretic and diuretic substances in the serum, as suggested by Lloyd and Lobotosky (14). As shown, the variation is too great to be ascribed to inconsistencies in the assay method.

In any case, alterations in the level of activity do not seem to be responsible for the retention of water associated with congestive heart failure, nor has the degree of liver impairment been shown to affect the level of activity in the serum. This is in contrast to the finding of Dochios and Dreifus (3), who reported increased amounts of antidiuretic substance, which were correlated with the degree of clinical edema, in the urine of such patients. This suggests that serum antidiuretic substance, besides differing from Pitressin in not being chloruretic, is also not identical with urinary antidiuretic substance.

SUMMARY

It has been demonstrated that human serum contains a labile substance which, when injected into hydrated rats, exerts an antidiuretic effect, which may vary with the extent of hydration. The antidiuretic activity of the sera of a group of 10 patients with congestive heart failure was compared with that of a normal group. No significant difference in antidiuretic activity was demonstrated. Nine subjects with liver disease also showed values within the normal range. It was concluded that the alterations in the serum level of antidiuretic activity were not responsible for the water retention associated with congestive heart failure and liver disease. The sera of all cases studied were shown to be non-chloruretic, thus differing from the antidiuretic principle present in posterior pituitary extracts.

Acknowledgments

This study was supported by a grant from the National Research Council of Canada to W.F.P. Dr. J. Doupe's helpful advice is gratefully acknowledged.

REFERENCES

1. Robinson, F. H., Jr., and Farr, L. E.: The relation between clinical edema and the excretion of an antidiuretic substance in the urine, *Ann. Int. Med.* **14:** 42–54 (July) 1940.
2. Bercu, B. A.; Rokaw, S. N., and Mussie, E.: An antidiuretic substance in the urine of patients with cardiac failure, *J. Lab. & Clin. Med.* **34:** 1585 (Nov.) 1949.
3. Dochios, M., and Dreifus, L. S.: Antidiuretic hormone studies in patients presenting edema, *Am. J. M. Sc.* **222:** 538–543 (Nov.) 1951.
4. Eversole, W. J.; Birnie, J. H., and Gaunt, R.: Inactivation of posterior pituitary antidiuretic hormone by the liver, *Endocrinology* **45:** 378–382 (Oct.) 1949.
5. Eversole, W. J.; Birnie J. H., and Gaunt, R.: Inactivation of posterior pituitary antidiuretic hormone by the liver, (abstract) *J. Clin. Endocrinol.* **8:** 616 (July) 1948.
6. Ralli, E. P.; Robson, J. S.; Clarke, D., and Hoagland, C. L.: Factors influencing ascites in patients with cirrhosis of the liver, *J. Clin. Investigation* **24:** 316–325 (May) 1945.
7. Hall. C. A.; Frame, B., and Drill, V. A.: Renal excretion of water and antidiuretic substances in patients with hepatic cirrhosis and rats with dietary liver injury, *Endocrinology* **44:** 76–82 (Jan.) 1949.
8. Birnie, J. H.; Eversole, W. J.; Boss, W. R.; Osborn, C. M., and Gaunt, R.: An antidiuretic substance in the blood of normal and adrenalectomized rats, *Endocrinology* **47:** 1–12 (July) 1950.
9. Birnie, J. H.; Jenkins, R.; Eversole, W. J., and Gaunt, R.: An antidiuretic substance in the blood of normal and adrenalectomized rats, *Proc. Soc. Exper. Biol & Med.* **70:** 83–85 (Jan.) 1949.
10. Hawk, P. B.; Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, ed. 12. Toronto, The Blakiston Co., 1947.
11. Dicker, S. E., and Ginsberg, M.: Some observations on the antidiuretic activity of rat serum, *Brit. J. Pharmacol.* **5:** 497–504 (Dec.) 1950.
12. Gaunt, R.: The adrenal cortex in salt and water metabolism, *in* Recent Progress in Hormone Research. New York, The Academic Press Inc., 1951, vol. 6, p. 247.
13. Lloyd, C. W., and Lobotsky, Julia: Serum antidiuretic substances and urinary corticosteroid in the human, *J. Clin. Endocrinol.* **10:** 318–329 (Mar.) 1950.

THE EFFECT OF INTRAVENOUS ACTH, WITH AND WITHOUT EPINEPHRINE

J. R. MARTIN, M.D. AND C. J. PATTEE, M.D.*

From the Clinical Investigation Unit, Queen Mary Veterans Hospital, Montreal, Canada

ACTH, when given intravenously, is capable of stimulating the adrenal gland. Renold *et al.* (1) found that if 20 mg. of ACTH is given intravenously for eight hours, the adrenal cortex is stimulated for a further eight to twelve hours. Sonenberg *et al.* (2), using labelled ACTH, showed in experimental animals that there was a very high concentration of ACTH in the adrenal glands if the animals were killed a few minutes after injection, but two hours later no ACTH was detectable in the adrenal cortices. Greenspan *et al.* (3) and Sayers (4) confirmed the rapid destruction or inactivation of ACTH when used in this manner.

McIntosh and Holmes (5) concluded that 10 mg. of ACTH given over twenty-four hours produced a maximum eosinopenia and increase in 17-ketosteroid excretion. Mandel and his workers (6) believe that there is symptomatic relief proportional to the duration of the intravenous infusion.

Long (7) has shown that epinephrine is capable of stimulating the anterior pituitary to secrete ACTH. Cope *et al.* (8) were unable to maintain adrenal cortical stimulation when epinephrine was given following a course of intramuscular ACTH. Other workers (9, 10), using comparable doses of epinephrine in oil, have noted no change in the symptoms or in the 17-ketosteroid excretion.

MATERIAL AND METHODS

The patients selected had rheumatoid arthritis. Patients 1, 2 and 3 were given 2,000 cc. of 5 per cent glucose and water intravenously over a twenty-four hour period. One week later this was repeated with the addition of 20 mg. of ACTH to the solution.

Patients 2–7 were given two courses of intravenous ACTH separated by one week. A dose of 1 cc. of 1:500 epinephrine in oil was given intramuscularly at three, eleven, and nineteen hours after the completion of the second intravenous infusion; as an added control, 1 cc. of peanut oil was

Received for publication May 24, 1952.

* Director.

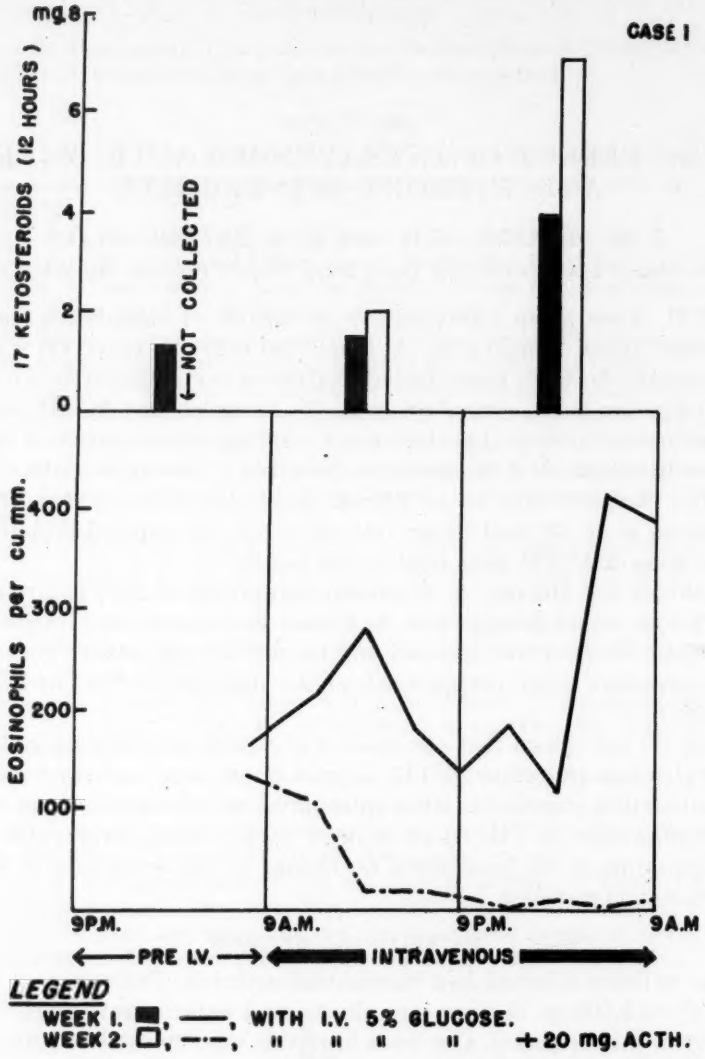


FIG. 1. Intravenous 5 per cent glucose followed by intravenous ACTH.

given intramuscularly at the same time intervals after the first infusion in Patients 5–7.

Eosinophil counts were made by the method described by Rud (11). The 17-ketosteroids in the urine were estimated by the method described by Callow *et al.* (12).

Figures 1–4 show examples of each group of patients.

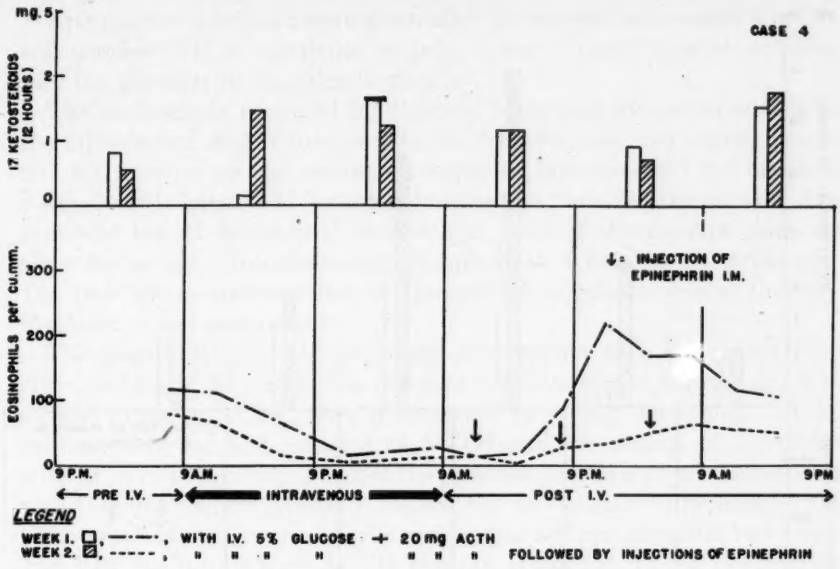


FIG. 2. Intravenous ACTH followed by intravenous ACTH plus epinephrine injections.

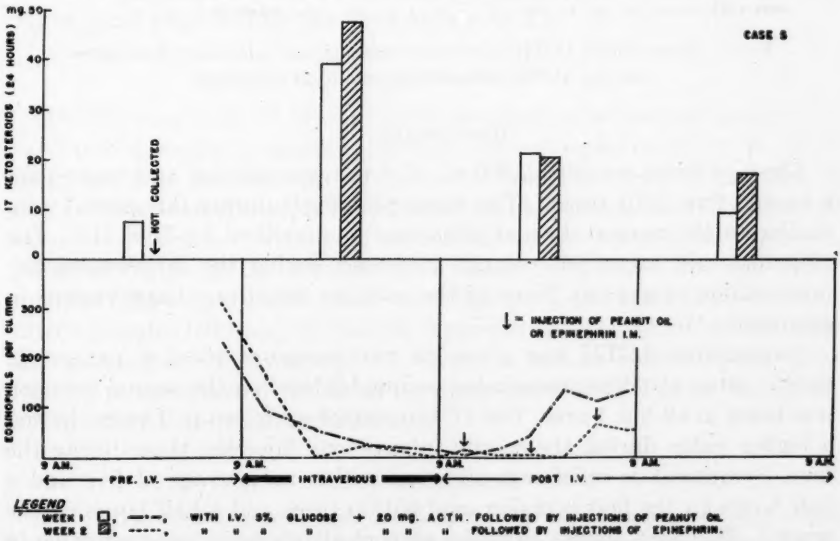


FIG. 3. Intravenous ACTH followed by peanut oil injections; then intravenous ACTH followed by epinephrine injections.

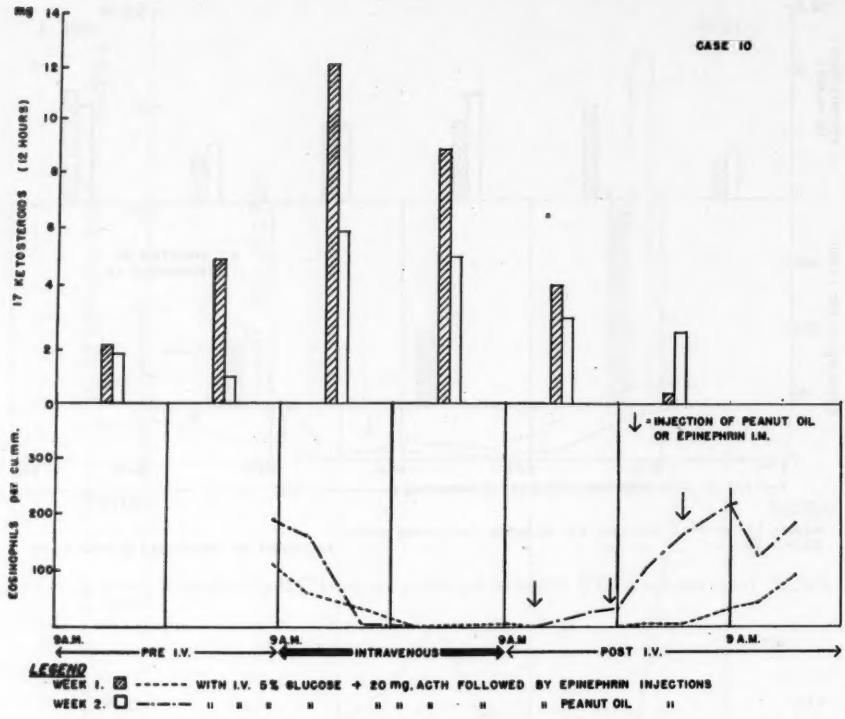


FIG. 4. Intravenous ACTH followed by epinephrine injections; then intravenous ACTH followed by peanut oil injections.

DISCUSSION

Three patients received 2,000 cc. of 5 per cent glucose and water over a twenty-four hour period. The eosinophil levels during this period were similar to the normal diurnal variations as described by Rud (11). The 17-ketosteroid excretion was not increased during the intravenous administration of glucose. None of the patients noted any improvement in symptoms.

Intravenous ACTH was given on two occasions to each patient at weekly intervals. The pre-infusion eosinophil level on the second occasion was lower in all but 1 case. The 17-ketosteroid excretion in 3 cases showed a higher value during the second intravenous injection than during the first. Symptomatic relief was observed within an average of five and a half hours on the first occasion, and within three and a half hours on the second. This may signify that the adrenal glands were more sensitive to the intravenous ACTH on the second occasion.

Epinephrine injections were given after the second intravenous administration of ACTH in 5 patients, in order to see if it were possible to maintain the stimulus to the adrenal glands.

The eosinopenia persisted for thirteen hours and fifteen minutes after the intravenous ACTH injection on the first occasion, and eighteen hours and ten minutes on the second. Epinephrine injections did not maintain a significantly greater 17-ketosteroid excretion than did the control. The generally low 17-ketosteroid excretion in most of the patients does not allow for accurate interpretation. Symptomatic relief was not prolonged. The only demonstrable effect of epinephrine injections was in the continuation of the eosinopenia.

The possibility that the prolonged eosinopenia with the epinephrine injections might be due to the different response of the adrenal gland to a second dose of ACTH, was investigated by giving the epinephrine injections after the first infusion of ACTH and the peanut oil injections after the second infusion. Three of the patients showed a longer eosinopenia with the epinephrine injections. There was no constant difference in the 17-ketosteroid output on either occasion, and the symptomatic relief produced by the intravenous ACTH was not prolonged by the epinephrine injections.

It was concluded that the epinephrine injections were responsible for the prolonged eosinopenia as compared with the control, but they had no effect in increasing the 17-ketosteroid output or continuing the symptomatic relief produced by the intravenous ACTH.

CONCLUSION

ACTH, in a dose of 20 mg. given intravenously over a twenty-four-hour period, caused a significant drop in the eosinophil count by the ninth hour, but this time was probably several hours in excess of the time of onset of the eosinopenia. ACTH caused an increase in the 17-ketosteroid excretion, which was maximum on the day of the intravenous injection. Relief of symptoms occurred, on the average, after four hours and thirty minutes following the first intravenous injection, and after two hours and fifteen minutes following the second. Epinephrine injected intramuscularly did maintain a lower eosinophil level but it had no effect in prolonging the symptomatic relief produced by intravenous ACTH or in causing an increased 17-ketosteroid excretion.

Acknowledgment

The authors wish to express their thanks to the nursing and laboratory staff of the Queen Mary Veterans Hospital, to Mr. M. Hearns for the charts and to Professor J. S. L. Browne, consultant of the Unit, for his criticisms and advice in this project.

REFERENCES

1. RENOLD, A. E.; FORSHAM, P. H.; MAISTERRENA, J., and THORN, G. W.: Intravenously administered ACTH, *New England J. Med.* **244:** 796–798 (May 24) 1951.
2. SONENBERG, M.; KESTON, A. S., and MONEY, W. L.: Tracer studies with labelled preparations of anterior pituitary hormones; ACTH, (abstract) *J. Clin. Endocrinol.* **10:** 809 (July) 1950.
3. GREENSPAN, F. S.; LI, C. H., and EVANS, H. M.: Disappearance rate of adrenocorticotropic hormone from rat's plasma after intravenous injections, *Endocrinology* **46:** 261–264 (March) 1950.
4. SAYERS, G.; BURNS, T. W.; TYLER, F. H.; JAGER, B. V.; SCHWARTZ, T. B.; SMITH, E. L.; SAMUELS, L. T., and DAVENPORT, H. W.: Metabolic actions and fate of intravenously administered adrenocorticotropic hormone in man, *J. Clin. Endocrinol.* **9:** 593–614 (July) 1949.
5. MCINTOSH, H. W., and HOLMES, C. B.: The intravenous use of ACTH, *Canad. M.A.J.* **65:** 33–35 (July) 1951.
6. MANDEL, W.; SINGER, M. J.; GUDMUNDSON, H.R.; WEISTER, L., and MODERN, F. W. S.: Intravenous use of pituitary adrenocorticotropic hormone (ACTH), *J.A.M.A.* **146:** 546–551 (June 9) 1951.
7. LONG, C. N. H.: Recent studies on function of adrenal cortex, *Bull. New York Acad. Med.* **23:** 260–282 (May) 1947.
8. COPE, C. L.; BOYSEN, X., and MCCRAE, S.: Some observations on endogenous cortisone excretion in man, *Brit. M. J.* **2:** 762–767 (Sept. 29) 1951.
9. DRESNER, E.; PUGH, L. G. C., and WILD, J. B.: ACTH. in rheumatoid arthritis compared with intramuscular adrenaline and with deoxycortone and ascorbic acid, *Lancet* **1:** 1149–1153 (June 24) 1950.
10. RECANT, L.; HUME D. M.; FORSHAM, P. H., and THORN, G. W.; Studies on the effect of epinephrine on the pituitary-adrenocortical system, *J. Clin. Endocrinol.* **10:** 187–229 (Feb.) 1950.
11. RUD, F.: Eosinophil count in health and in mental disease; biometrical study, *Acta psychiat. et neurol.*, Supp. 40, pp. 1–443, 1947.
12. CALLOW, N. H.; CALLOW, R. K., and EMMENS, C. W.: Colorimetric determination of substances containing grouping—$CH_2 \cdot CO$—in urine extracts as indication of androgen content, *Biochem. J.* **32:** 1312–1331 (Aug.) 1938.
13. THORN, G. W.: Diagnosis and Treatment of Adrenal Insufficiency. A monograph in American Lectures in Endocrinology edited by W. O. Thompson. Springfield, Illinois, Charles C Thomas, Publisher, 1949.
14. MCINTOSH, H. W., and HOLMES, C. B.: Some evidence suggesting the suppression of adrenocortical function by cortisone, *Lancet* **2:** 1061–1064 (Dec. 8) 1951.

A SYNDROME CHARACTERIZED BY ESTROGENIC INSUFFICIENCY, GALACTORRHEA AND DECREASED URINARY GONADOTROPIN*

J. ARGONZ, M.D.† AND E. B. DEL CASTILLO, M.D.‡

From the Service of Endocrine Diseases of the Rivadavia Hospital (Ward 16), Buenos Aires, Argentina

IN 1932, Ahumada and del Castillo (1) published the history of a patient presenting galactorrhea and amenorrhea with symptoms of estrogenic insufficiency and absence of urinary gonadotropins. To this observation, the first to our knowledge, other studies made during the last years in the Endocrinology Service of the Rivadavia Hospital have been added. In these new cases the existence was verified of galactorrhea with amenorrhea and urinary gonadotropin (FSH) decreased below 6 mouse units in twenty-four hours.

At this stage of our observations we were informed that at the meeting of the Association for the Study of Internal Secretions held in Atlantic City in June 1951, a communication was presented by Forbes, Henneman, Griswold and Albright under the title "A syndrome, distinct from acromegaly, characterized by spontaneous lactation, amenorrhea, and low follicle-stimulating hormone excretion." Of this paper, we know only the abstract published in the July 1951 issue of *The Journal of Clinical Endocrinology* (2).

We shall refer to only 4 such cases that we have had the opportunity to observe. We consider that the symptomatology presented by them coincides with that which characterizes the patients referred to by Ahumada and del Castillo (1932) and by Forbes *et al.* (1951).

REPORT OF CASES

Case 1

N. P., an 18-year-old, single girl, was seen by us complaining of secondary amenorrhea and a milky secretion from the mammary glands. These disturbances began one year before observation. She complained of headache and her weight had increased 4 Kg. since the onset of her disease. During the year of evolution of her amenorrhea she had two menstrual periods, under estrogen therapy.

Received for publication June 4, 1952.

* This investigation was aided by a grant from E. R. Squibb & Sons, S. A., Argentina Research Laboratories.

† Present address: Montevideo 711, Buenos Aires, Argentina.

‡ Present address: Charcas 2429, Buenos Aires, Argentina.

The family history was of no interest; the personal history showed a normal infancy, although she was always considered weak up to the time of puberty. She had never been pregnant. The first menstruation appeared at the age of 12 and, until the onset of the present disease, her cycles were of the 4/30 type.

Physical examination: The patient was 156 cm. tall and was well nourished; axillary and pubic hair were consistent with sex and age; the breasts were of normal size and of firm and homogeneous consistency; nipples and areolae were small and scantily pigmented; there was no evidence of Montgomery's corpuscles or of visible venous circulation. By moderate compression, a milky secretion came from the breasts in a filiform spurt, it being possible with this procedure to extract several cubic centimeters from each gland.

Genitals—The pubis was of normal appearance, although small, and the vulva was slightly hypoplastic. The labia minora: short and scarcely pigmented on the left, better developed and well pigmented on the right. The uterus was small and in a position of hyperanteflexion and moderate retroversion. The left adnexae were hard and small; the right adnexae were not palpable.

Laboratory examination: The urine was normal. The concentration of blood urea was 5, sugar 83, and cholesterol (Grigaut) 220 mg. per cent. The hemogram was normal. The Aschheim-Zondek test for gonadotropin in the urine gave negative results. The Fluhman procedure for gonadotropin in blood also gave negative results, as did Frank's technique for follicular hormone in the blood. The basal metabolism was +8 per cent. (These determinations were made during the years 1931–1932.) The examination of the mammary secretion showed that it was milk. The sella turcica was normal on roentgenologic examination. Ocular fundi and visual fields were normal.

Summary: This patient was of normal proportions and without any apparent maldevelopment of her sexual characteristics. She had secondary amenorrhea and galactorrhea of one year's duration. In her genital apparatus the only abnormality was her small uterus. The mammary glands were of medium size, firm, and without nodules, but the nipples and areolae were small and scantily pigmented.

Case 2

I.L. de P., a 33-year-old married woman, consulted us because of amenorrhea and because, more than a year before, she had noted a milky secretion of the breasts. Although always slightly obese, the increase in weight had become accentuated during the previous year. She complained of intermittent, sudden hot flushes. Sometimes she suffered from insomnia and a desire to cry. She felt nervous and had a sensation of formication in the face and hands.

The family history was of no interest. In the personal history the following data were found: normal development during childhood; at the age of 13 the secondary sexual characteristics (breasts, pubic and axillary hair) developed, but the menarche did not take place. At the age of 15 she had an appendectomy and at 16, malaria. She married a healthy man at the age of 23, but never became pregnant.

Physical examination: The patient was a well nourished woman with an increase of fatty tissue in the abdominal and pelvic girdles. Her height was 150 cm. and her weight 67 Kg. She had abundant, thick scalp hair and abundant axillary hair. Pubic hair was fairly abundant, with prolongation towards the navel. There was excess hair on the lower limbs, less on the arms, and very little on the face. The right breast was well developed, of elastic consistency, without nodules, and the areola and nipple were of corresponding size. The left breast was much smaller, but had similar characteristics.

By pressing the gland manually, a milky secretion was obtained, more marked on the right side. The patient stated that many days her brassiere was wet because of the spontaneous secretion of her breasts.

Genitals—The vulva and vagina were like those of an adult person; they showed an abundant whitish discharge and small red spots in the mucosa of the vaginal neck. The neck of the uterus was small, of conical shape, hard, and non-tender when displaced. The body of the uterus was very small and in a position of anteversoflexion. Palpation revealed a left ovary, increased in size and somewhat tender.

Laboratory examination: Determinations of urinary gonadotropin were performed on four different occasions, with uniformly negative results for 6 m.u. (mouse units). Lateral radiography of the skull showed a normal sella turcica. The determination of 17-ketosteroids gave 17.08 mg. in twenty-four hours. The hemogram was normal. Blood sugar concentration was 87 mg. per cent, and the value for blood carotenes was 1.5 U. Ocular fundi were normal. A scanty biopsy specimen of the endometrium showed lack of mucosa; in a subsequent curettage specimen, scanty endometrium of the atrophic type was found.

Summary: This slightly obese woman suffered from primary amenorrhea, but had normal development of secondary sexual characteristics. Her right breast was larger than her left. For more than a year she had noted a milky secretion from the breasts, both spontaneously and with manual pressure. The uterus was very small, and the urinary and vaginal smears showed a hypoestrogenic picture. The determination of urinary gonadotropin on repeated occasions always gave values below 6 m.u. per twenty-four hours. Biopsy of the endometrium showed the presence of a small amount of mucosa of the atrophic type.

Case 3

E.A. de A., a 36-year-old married woman, came for consultation because of secondary amenorrhea of several years' duration, accompanied for the last three years by galactorrhea. She had been treated with estrogens; occasionally menstruation occurred, but reappeared only under further therapy. In the previous year she had suffered in both breasts from repeated attacks of mastitis, which were sometimes associated with fever and required antibiotic treatment.

Her family and personal histories, until the beginning of her married life (fifteen years before), were not pertinent to her present disease. The menarche appeared at the age of 12, with normal development of the secondary sexual characteristics, the menstrual cycles occurring at regular intervals and being of normal duration. After marrying, she increased markedly in weight and the menstrual intervals became prolonged, menstruation being absent sometimes for periods of from one to two months. In the fifth year of marriage she had her only pregnancy, which ended in normal parturition. After childbirth her weight increased to over 80 Kg.; her weight before marriage was 48 Kg. She observed occasional delay in her menstrual periods. This was the situation for six years, but in the last three of these years the menstrual disturbances were accentuated and the amenorrhea which developed yielded only to medication. Almost at this same time she began to notice a spontaneous milky secretion with mastalgia, first in the right breast and then in the left; there were acute attacks of lymphangitis. The lymphangitis, starting from the nipples, formed a wide, indurated plaque. This process was apparently due to infection, caused by the milking maneuvers performed by the patient in an effort to eliminate the troublesome galactorrhea.

Physical examination: Her height was 162 cm. and her weight was 84 Kg. Pubic and

axillary hair was well developed and had normal female distribution. The breasts were large, pendulous, and of a somewhat flaccid consistency; the glands could be palpated and some nodules were present. At the time of the first examination there was an acute lymphangitis, with the characteristics just described. The nipples and areolae were of a dark brown color, which we attributed to the synthetic estrogen (diethylstilbestrol) therapy. The patient's clothes were dampened by the spontaneous secretion of the breasts. By manual pressure she obtained a milk-like secretion.

Genitals—The vulva was of normal appearance; the vagina was without pathologic peculiarities, with a normal-appearing neck. The uterus was somewhat small for a primipara. Palpation of the adnexae showed them to be of normal size and consistency.

Laboratory examination: Determination of urinary gonadotropin gave values below 6 m.u. per twenty-four hours. Examination of the urinary sediment showed a hypoestrogenic picture; there was a predominance of superficial spinous cells with a large nucleus but some deep spinous cells were also present. Urinalysis and a hemogram gave normal results. Radiography showed the sella turcica to be normal. The ocular fundi were normal.

Summary: This patient showed a secondary amenorrhea with galactorrhea, appearing the sixth year after her only childbirth; no abnormality was noted immediately following the birth. Smears of the urinary sediment showed reduction of the circulating estrogens. The genital examination did not provide any clue to the condition, except that the uterus was somewhat small for a primipara. The biologic determination of the urinary gonadotropins gave values below 6 m.u. per twenty-four hours. The breast condition was several times accompanied by lymphangitis and fever.

Case 4

F. G., was a 29-year-old, single woman. Thirteen years before, the presence of galactorrhea upon mammary pressure had been observed during an examination. A year before the present visit, we had found galactorrhea in both breasts. The patient stated that in the long interval which elapsed since the first observation thirteen years before, the condition had not worried her and the secretion was never spontaneous. The menarche appeared at the age of 13, with regular, nonpainful cycles of the 5/30 type. She had never had menstrual disturbances until the previous three years, when they suddenly began; the patient attributed them to a calcium injection and a blood withdrawal for autohemotherapy. She had not been sick previously, except for catarrhal bronchitis. She had had ocular treatment because of strabismus.

Physical examination: She was a well nourished and well developed woman. Her height was 154 cm. and her weight, 59 Kg. There was a divergent strabismus of the left eye. The breasts were well developed, turgid, and without nodules. The areolae and nipples were of normal size and coloring. By pressing both breasts, a white milky liquid spurted from the milk ducts.

Genitals—The vulva was normal. The labia majora were normal and the pigmented labia minora were well developed and of a dark brown color. The clitoris was of medium size (average) and its hood was somewhat large. The hymen was preserved. Upon rectal palpation the uterus was felt to be of normal size and shape; no adnexal masses were palpated.

Laboratory examination: The smear of the urinary sediment, repeated on several occasions, always gave a hypoestrogenic type of picture, corresponding to a relative estrogen insufficiency. Hemogram: RBC 4,700,000; WBC 5,000; neutrophils 74; lymphocytes 21; monocytes 4; and eosinophils 1 per cent. The assay of urinary gonadotropins

TABLE 1. SUMMARY OF THE CLINICAL HISTORIES

Initials, age (yrs.) & marital status	Clinical status	Mammary glands	Sexual characteristics	Urinary gonadotropins	Smears of urinary sediment	Other data
N.P.,* 18, single. *Case 1*	Secondary amneorrhea. Intense galactorrhea. Never pregnant.	Normal consistency. Small areolae and nipples. Milky secretion in filiform spurts.	Normal pubic and axillary hair. Small uterus. Left adnexa hard and small; right not palpated.	Absent.		Normal sella turcica and eye fundi. Cholesterolemia, 220 mg./100 cc. B.M.R. +8%.
J.L. de P., 33, married. *Case 2*	Primary amenorrhea and galactorrhea of 5 years' duration. Slight obesity. Never pregnant.	Well developed, larger on the right. Milky secretion.	Fairly abundant pubic hair. Normal axillary hair. Very small uterus. Left adnexa somewhat enlarged; right normal.	Negative for 6 m.u. Repeated determinations.	Hypoestrogenic	Normal sella turcica and eye fundi. Carotenes: 1.5 U. 17-Ketosteroids: 17.08 mg./24 hrs. Endometrium atrophic. Hirsutism.
E.A. de A., 36, married. *Case 3*	Secondary amenorrhea and galactorrhea for 3 years. Mastitis. Postmarital obesity. A pregnancy 9 years before.	Well developed. Sometimes a milky secretion and at other times a sanguinous colostrum.	Normal pubic and axillary hair. Small uterus. Normal adnexae.	Negative for 6 m.u.	Hypoestrogenic	Normal sella turcica, eye fundi and visual fields. Mammary lymphangitis.
F.G., 29, single. *Case 4*	Galactorrhea for 13 years. Amenorrhea for 1 year. Virgin.	Well developed. On pressure, a milky secretion.	Normal pubic and axillary hair. Uterus of normal size. Normal adnexae.	Negative for 6 m.u.	Hypoestrogenic	Normal sella turcica, eye fundi and visual fields. Discrete obesity.

* Observation already published by Ahumada and del Castillo in 1932 (1).

was negative for 6 m.u. per twenty-four hours. Radiography showed the sella turcica to be normal. Ocular fundi and visual fields were normal.

Summary: This patient, in whom the existence of galactorrhea without any other symptomatology, was found thirteen years previously (at the age of 16), suffered from menstrual disturbances and had been amenorrheic for a year. The galactorrhea persisted and urinary gonadotropins showed values below 6 m.u. per twenty-four hours. The amenorrhea and galactorrhea were not influenced by serial gonadotropin, estrogen and progesterone therapy.

DISCUSSION

In the 4 patients presented here (Table 1), there was persistent galactorrhea, which was not modified by the treatment given. The intensity of this symptom varied from the extrusion of a few drops of whitish secretion up to the abundant and spontaneous elimination of milk. The size of the breasts and the characteristics of the nipples and areolae appeared to be normal. The amenorrhea was primary in 1 case and secondary in the other 3. With the exception of Case 3, no related data of interest were found in the menstrual histories of the secondary cases. In Case 3, coinciding with the obesity after marriage, oligomenorrhea appeared until the onset of the clinical picture that we are now studying.

The gynecologic examinations showed a uterus which was of normal size in 1 case and small in 3 cases, without other modifications and with maintenance of normal secondary sexual characteristics. The study of the urinary sediments and vaginal smears showed an accentuated hypoestrogenic type of picture.

The values for urinary gonadotropin were always below 6 m.u. per twenty-four hours. Because of these results, the determinations were repeated in some cases, as our tentative diagnosis had been precocious menopause, and we had expected to find values above normal. In Case 3, a recent determination made after a year of estrogenic therapy, showed a value below 6 m.u., which indicates the lack of response to the adenohypophysis.

Taking into consideration the three main symptoms characterizing this syndrome—amenorrhea, galactorrhea and low urinary gonadotropin—it is important to establish the differential diagnosis when comparing these cases with other pathologic processes in which the same symptoms are observed.

Galactorrhea with amenorrhea has been found after castration and in precocious and physiologic menopauses. In these three conditions, the determination of urinary gonadotropin gives values above normal—higher than 96 m.u. per twenty-four hours—as we reported in 1948 (de la Balze and Argonz (3)).

Another process showing a close similarity to this new syndrome is

Chiari-Frommel's. Galactorrhea with amenorrhea is found in this condition, the urinary gonadotropin being either absent (as in the 2 cases presented by Pasqualini, Gimenéz and Jassim (4)) or below 6 m.u. per twenty-four hours (as in the case recently studied on our Service). The fundamental differentiation from this process lies in the etiology, since the Chiari-Frommel syndrome always appears after childbirth and is strictly related to it. Besides, it is generally accompanied by a deficient nutritional state and by psychic disturbances characterized by neurosis or psychoneurosis.

Our patient showed the picture of a psychosis with hallucinations, which later improved. It is worthy of note that this patient maintained the symptomatology of galactorrhea, amenorrhea and low values for FSH for more than six years after parturition, and in spite of numerous and varied treatments.

Another condition presenting galactorrhea with amenorrhea is acromegaly. One of the first observations in our country was that of del Castillo and Lanari (5), who published in 1933 the case of a virgin suffering from acromegaly, in whom the aforementioned clinical picture was observed. In the publication of de la Balze and Argonz (3) concerning results of the determination of hypophysial gonadotropins in amenorrheas, 2 cases of acromegaly with values for urinary gonadotropin below 6 m.u. are included. Therefore, the three principal symptoms are also found in this process, but the rich symptomatology of acromegaly makes the differential diagnosis easy.

On several occasions we have observed, on our Service, the association of amenorrhea with galactorrhea and low values for urinary gonadotropin in cases of tumors effecting the adenohypophysis or the adjacent structures of the brain or its coverings. In these conditions, alterations of vision by pressure on the optic chiasm, radiologic evidence of modifications in the sella turcica, and sometimes symptoms of endocranial hypertension, point to the right diagnosis.

The presence of galactorrhea with amenorrhea and low values for gonadotropin has been mentioned in Simmonds' disease. In these cases the global insufficiency of the adenohypophysis, with its clinical and humoral manifestations, should be taken into account in the differential diagnosis.

We have indicated the principal pathologic processes which it is necessary to consider in order to delimit with precision the diagnosis of this new syndrome; it remains to comment on its physiopathogenesis. We can only offer an hypothesis, because the etiology is not known.

The factors related to the milky secretion in the human species still present many obscure points in spite of intense study in many laboratories. The important role of the adenohypophysis, and especially of one of its

hormones, prolactin, is well known. As a summary of the numerous publications, we may say with Folley and Young (6) that lactation is the result of the action of a hormonal complex within which prolactin and adrenocorticotropin stand out as very important factors.

In the cases we have presented here, the milky secretion was not related to pregnancy and childbirth, since it occurred in nulliparas, or else the disturbance began several years after the last gestation (Case 3). Nevertheless, it is certain that here, as in normal lactation, there intervenes an important factor, such as the *modification of the estrogenic level*, which would unchain the mechanism of milk production. There is justification for the suggestion that there is an estrogenic threshold for the stimulus of the hypophysis (probably inferior to another inhibition threshold) which governs the physiologic changes—the stimulus or the inhibition predominating according to the level of the circulating estrogens.

In all the patients observed, there was always found an estrogenic deficiency; this condition would stimulate the hypophysis to produce hormonal lactogenic and galactopoietic factors.

It is interesting that Forbes *et al.* (2) although they do not offer a physiopathologic explanation of the syndrome, seem to give importance to hyperfunction of a sector of the adenohypophysis—that of the eosinophil cells. There are some reports showing that normally these cells segregate the prolactin (*e.g.*, Schooley and Riddle (7)). Everything seems to indicate that this syndrome is hypophysial or hypothalamo-hypophysial, because if it were of gonadal origin, there would be an increase of FSH such as occurs in cases of castration and the menopause. Thus the estrogenic insufficiency would be secondary, provoked by the lack of hypophysial stimulus. In conjunction with the insufficiency in the gonadotropin sector of the hypophysis, the function of other elements would be stimulated (probably that corresponding to the eosinophil cells); this would increase the production of prolactin and adrenocorticotropin, thus starting the galactorrhea.

In none of our observations have we been able to trace any present or past process related to disturbances of the hypothalamus; neither the radiograms of the region nor the examination, gave any evidence of such changes.

We would be inclined to suggest as an anatomic basis for this syndrome an *eosinophilic hyperfunction of the anterior lobe of the hypophysis*. As has already been said, one of the fundamental symptoms of the syndrome is the amenorrhea, usually secondary. The results of the determination of FSH in the urine indicate that it is an amenorrhea of hypophysial origin. The symptoms of ovarian insufficiency are dependent on this. The galactorrhea or spontaneous milky secretion, not connected naturally with pregnancy or with treatment, suggests a hyperfunction of the anterior lobe

of the hypophysis in the sector of its eosinophil cells, which are supposed (with sound reasons) to stimulate the production of prolactin. It cannot be stated that our patients had an adenoma or a hyperplasia of the eosinophil cells. Besides, there are hypophysial tumors classified as chromophobe, which are accompanied by galactorrhea; and it is probable that they are cases similar to the ones just presented.

Therefore, it is logical to speak of two syndromes related to the anterohypophysial eosinophil cells, namely, the acromegalic syndrome and the one which is the object of the present paper. The decrease of the urinary FSH may be due to the upset of the equilibrium with the prolactin. We do not know if, in this syndrome, the hypothalamus is injured or altered in its function; neither do we know if it may have any action on the secretion of prolactin.

SUMMARY

Four patients are presented who showed a syndrome characterized by galactorrhea, amenorrhea and low values for urinary gonadotropin. This symptomatology had no relation to pregnancy.

In all the patients evidences of estrogenic insufficiency were found, but substitution therapy modified the clinical picture only temporarily.

The syndrome appears to be of hypophysial origin, perhaps produced by an upset of the hormonal balance between FSH and prolactin. The possible intervention of the hypothalamus remains an unknown factor.

REFERENCES

1. Ahumada, J. C., and del Castillo, E. B.: Amenorrea y galactorrhea, *Bol. Soc. gin. y obst.* **11**: 64, 1932.
2. Forbes, A. P.; Henneman, P. H.; Griswold, G. C., and Albright, F.: A syndrome, distinct from acromegaly, characterized by spontaneous lactation, amenorrhea, and low follicle-stimulating hormone excretion, (abstract) *J. Clin. Endocrinol.* **11**: 749, 1951.
3. de la Balze, F. A., and Argonz, J.: El dosaje de gonadotrofinas hipofisarias en la orina, *Medicina* **8**: 383, 1948.
4. Pasqualini, R.; Giménez, L., and Jassim, A.: Interpretación endócrina del sindrome de Chiari-Frommel, *Rev. Asoc. méd. argent.* **65**: 219, 1951.
5. del Castillo, E. B., and Lanari, A.: Sindrome caracterizado por crecimiento exagerado, amenorrea y galactorrea, *Semana méd.* **2**: 303, 1933.
6. Folley, S. J., and Young, F. G.: The galactopoietic action of pituitary extracts in lactating cows, *J. Endocrinol.* **4**: 194, 1945.
7. Schooley, J. P., and Riddle, O.: The morphological basis of pituitary function in pigeons, *Am. J. Anat.* **62**: 313, 1938.

FACTORS GOVERNING THE DEVELOPMENT OF THE CHICK EMBRYO THYROID. I. DETERMINATION OF THE TIME AT WHICH I^{131} COLLECTION BEGINS*†

J. B. TRUNNELL, M.D. AND F. T. BRAYER, M.D.

From the Section of Experimental Medicine, University of Texas, M. D. Anderson Hospital for Cancer Research, Houston, Texas

THE results of therapy of thyroid cancer with radioactive iodine have, in general, been disappointing. Despite attempts to improve over-all uptake and homogeneity of distribution by pretreatment with thyroidectomy, thiouracil or the thyrotropic hormone, no cures have been obtained (1). The major barrier to success has been the spotty nature of iodine concentration throughout a given thyroid cancer. Although exceptions have been encountered, the areas of good uptake have generally corresponded to the areas of greatest differentiation, whereas the portions of tumor that are poorly differentiated have failed to concentrate significant quantities of the isotope (2). Our attention was turned toward a study of the factors regulating the differentiation of embryonic thyroid tissue in the hope that information obtained in such studies might eventually be utilized to good advantage in preparing thyroid cancers for treatment with radioactive iodine.

This report presents and discusses briefly data concerning the time at which the thyroid first begins to concentrate radioactive iodine in the normal chick embryo. Also presented are data obtained from embryos which have been pretreated with the thyrotropic hormone.

In the past, many different techniques have been utilized to attempt to ascertain the earliest stage at which the chick embryo thryoid first begins to function. As a result of studies by Bradway (3) (1929), it was concluded that thyroid function did not begin until the twelfth day of incubation, a point of view based upon the observation that no colloid could be seen until that time.

Small globules of colloid were seen by Hopkins (4) (1935), however, as

Received for publication September 25, 1952.

* Read at the Annual Meeting of the American Goiter Association, St. Louis, Missouri, May 1–3, 1952.

This article will be included in the bound volume of the 1952 "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1953.

† This work was supported in part by a grant from the American Cancer Society.

early as the tenth day. Furthermore, thyroid material from these ten-day chick embryos had the ability to bring about metamorphosis in tadpoles.

Coleman (5), working in Rawson's laboratory (1947), administered radioactive iodine to chick embryos and found the first significant uptake of radioactive iodine to occur between the tenth and eleventh days of incubation. Hansborough and Khan (6) reported (1951) a series of autoradiographic studies of the chick embryo thyroid, and concluded that the incorporation of I^{131} begins on the eleventh day of incubation.

MATERIALS AND METHODS

Fertilized eggs of a hybrid strain of New Hampshire Red and white leghorn chickens were obtained from the experimental farms of the Texas A. & M. College at College Station, Texas. These eggs were collected from the nests every three hours in order to minimize lack of uniformity in incubation time.

In the first series of experiments, designed to determine the most suitable portion of the egg into which radioiodine might be injected, 5 microcuries of I^{131} in 100 microliters of isotonic saline solution were injected into (a) the air chamber, (b) the yolk sac, and (c) onto the chorio-allantoic membrane of twelve-day-old embryos. Twenty-four hours later the thyroids were removed and the relative uptakes of iodine were determined. In this and in the succeeding three series of experiments, each value reported was obtained by averaging data from no fewer than 12 embryos. In the second, third, and fourth experiments, 5 microcuries of radioactive iodine were delivered from an accurately calibrated 100-microliter pipette directly onto the chorio-allantoic membrane, twenty-four hours in advance of determining uptake.

In Experiment II the thyroids were removed and counted for radioactivity individually, from the fifth through the thirteenth day of incubation. Uptake was measured not only in the extirpated thyroid itself but also in the remainder of the embryo. Embryos were removed from the liquid content of the egg by means of a perforated "spoon" and were rinsed once in clean isotonic saline solution, then blotted before being placed in previously weighed glass cylinders for reweighing and counting.

In Experiment III values were obtained for whole embryos (including the thyroid) from the fifth through the thirteenth day of incubation. In Experiment IV the circumstances of Experiment III were duplicated with the exception that 0.8 mg. of Armour thyrotropic hormone (Lot # J 13903R[1]) was introduced onto the chorio-allantoic membrane forty-eight

[1] In a subsequent "run," lot #K468808R was used with almost identical results. This material was supplied by Doctors John Mote and George Kamen of the Armour Laboratories, Chicago, Ill.

hours before the administration of I^{131} (seventy-two hours before counting). All counts were made with a Texas Company high sensitivity "toroidal" well counter.

RESULTS

Experiment I. The uptake figures obtained when I^{131} was introduced into the yolk sac varied considerably but were uniformly much lower than the values obtained following chorio-allantoic membrane administration. On the average only about one-fifth as much iodine was found in the thyroids of chicks which had received the isotope in the yolk sac, although in several instances values in the latter group approached those of the chorio-allantoic group. Administration into the air chamber yielded values which

TABLE 1. THE RELATIONSHIP BETWEEN AGE AND I^{131} UPTAKE OF EXTIRPATED THYROIDS AND OF THE RESIDUAL EMBRYOS

Age in days	Thyroids (average counts/min.)	Embryos (average counts/min.)
9	16	263
10	382	525
11	943	1,177
12	2,000	1,179
13	2,405	2,947
14	3,132	9,992
15	5,852	17,866

The selective concentration of I^{131} by the thyroid is observed first at the end of the tenth day of incubation. Note that at the age of 12 days the thyroid contains more iodine than the entire balance of the embryo.

were satisfactorily uniform but which were only about half as large as those obtained following chorio-allantoic membrane administration. For these reasons we chose the membrane route of administration in subsequent experiments.

Experiment II. Results are summarized in Table 1. On the ninth day of age the average number of counts per minute in the thyroid was only 16, whereas in the balance of the embryo the value obtained was 263. By the tenth day, however, the thyroid contained nearly half the total radioiodine in the embryo. On the twelfth day the thyroid actually contained more than the entire balance of the embryo. Thereafter, through the fifteenth day at least, even though the total amount in the thyroid increased steadily, the balance of the embryo, as a result of its rapidly increasing mass, contained significantly more than did the thyroid.

Experiment III. It was felt that since the thyroid is known to be the tissue most avid for iodine, a curve drawn from counts of the whole embryo

should show a sharp upswing on the day of beginning thyroid function. That such is the case is shown in Figure 1 (*see also* Table 2 for numerical values). Here it is apparent from consideration of the curve depicting the weight gain of the embryo that the increased concentration of radioactive iodine is not a function of the increasing mass of the embryo. The I^{131} uptake curve is seen to increase its slope between the tenth and eleventh days of the experimental period, whereas the weight curve remains smooth.

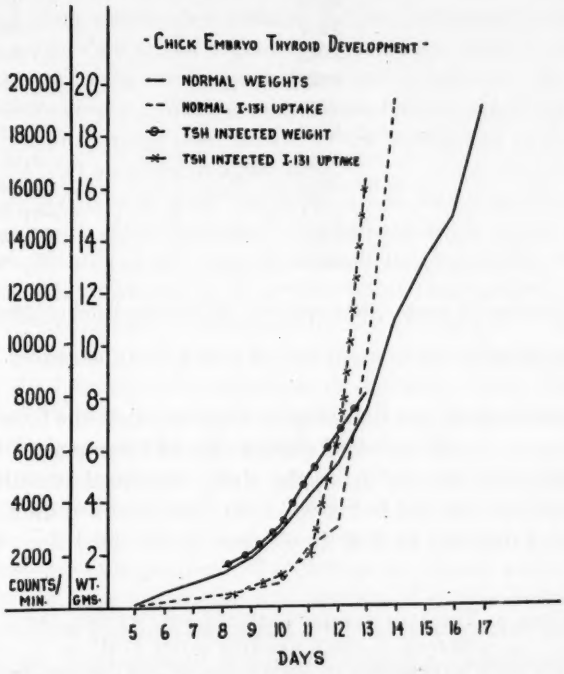


FIG. 1. Experiments III and IV are depicted graphically. Observe that although the growth curves of the embryos (both normal and TSH-injected) are smooth, there is a sudden change in the shape of the I^{131} content curves, beginning at the age of 10 days. Note further that after the age of 11 days the TSH-injected embryos collected more I^{131} than did the controls.

Experiment IV. Embryos obtained from chicks which had received thyrotropic hormone did not collect iodine sooner than did the uninjected controls. It will be seen, however, in Figure 1 (*see also* Table 3 for numerical values) that the total quantity collected was uniformly somewhat greater once function had begun. The fact that the TSH-injected embryo weights were slightly greater than those of the controls is probably the

TABLE 2. RELATIONSHIP BETWEEN AGE, WEIGHT, AND I^{131} UPTAKE IN NORMAL CHICK EMBRYOS*
(DATA FROM EXPERIMENT III, SHOWN GRAPHICALLY IN FIGURE 1.)

Age in days	Average weight in grams	Average I^{131}, counts/min.
5	0.1305	89
6	0.4888	161
7	0.8870	248
8	1.2967	281
9	1.7662	369
10	2.8323	864
11	4.2759	2,227
12	5.5562	3,053
13	7.6988	9,641
14	11.0333	19,620
15	13.2771	36,006
16	15.0967	57,026
17	19.3679	87,466

* μc. of I^{131} injected 24 hours before embryos were weighed and counted.

result of the presence of a small amount of growth hormone in the TSH preparation.

In all experiments it was consistently observed that the total iodine content of embryos which had died during the 24-hour period between I^{131} administration and removal from the shell, contained considerably more radioactive iodine than did living embryos. The factor of difference ranged from three and one half to five times more in the dead than in the living embryos.

TABLE 3. THE RELATIONSHIP BETWEEN AGE, WEIGHT, AND I^{131} UPTAKE IN CHICK EMBRYOS GIVEN TSH*
(DATA FROM EXPERIMENT IV, SHOWN GRAPHICALLY IN FIGURE 1.)

Age in days	Average weight in grams	Average I^{131}, counts/min.
8	1.4347	365
9	2.0749	520
10	3.0421	1,151
11	4.9852	1,904
12	6.6024	6,596
13	8.1747	16,775

* TSH (0.8 mg.) was injected 72–96 hours before embryos were weighed and counted. I^{131} (5 μc.) was injected 24 hours before embryos were weighed and counted.

DISCUSSION

The results obtained in these experiments agree with those recorded by Coleman and Rawson (5) and by Hansborough and Khan (6). Attention must be called, however, to the matter of terminology discussed by the latter authors, who prefer to speak of the age of the embryo in terms of the day of incubation *in progress*. Inasmuch as our program was arranged to harvest the embryos at the conclusion of any whole multiple of twenty-four hours, we speak of the length of the period of incubation *actually completed*. Hence their eleven-day embryo would correspond more nearly to our ten-day stage. One might argue equally well for either system (a child of 10 is of course living his eleventh year, but is labelled "10," a convention which influenced our choice); perhaps a third system (age in hours), alluded to by Hansborough and Khan, would be superior to either. At any rate their data and ours are in agreement.

The quantity of thyrotropic hormone which was administered to our embryos was large (approximately 3 Waleszek-Koch chick units). The fact that no hastening of the onset of iodine-collecting ability was observed suggests that this substance is not a very important factor in the process of thyroid differentiation, although naturally the fact that the TSH was not of chick origin must be taken into account. Fugo (7) (1940) showed in fact that in the hypophysectomized (at thirty-three hours) chick embryo, thyroid differentiation proceeded in essentially normal fashion, although it subsequently was not altogether maintained.

It is of interest, however, that the ability of the newly differentiated chick embryo thyroid to respond to TSH was evident in Experiment IV. By "respond" can be meant only the increased uptake of I^{131} observed, since obviously no measurement of iodine-metabolizing ability was made. Such studies are in progress. Autoradiograms of chromatograms of chick embryo thyroids at ages one day apart are being made in normal, hypophysectomized and TSH-treated groups of embryos. The results of these endeavors will be reported subsequently.

No detailed studies have been made of the phenomenon of the increased whole-embryo collection of I^{131}, which occurs if the embryo dies. Either diffusion into the embryo occurs more readily in the absence of membrane-discriminating powers present only in life, or there is fixation of iodine by denatured protein. Several years ago, in experiments conducted with Dr. William Smith, we found that I^{131} was taken up to a considerably greater degree by the necrotic central portions of tumors in mice than it was by the tumors or other viable tissues (except thyroid) themselves. There would appear to be some parallel between that situation and the one described here.

SUMMARY AND CONCLUSIONS

1. In the interest of uniformity of results and the use of small amounts of I^{131} per chick, the chorio-allantoic membrane route of administration appears preferable to that of either the yolk sac or the air space.

2. Selective concentration of I^{131} by the embryonic chick thyroid begins toward the end of the tenth day of incubation.

3. The administration of thyrotropic hormone does not alter the time at which the chick thyroid begins to collect I^{131}, but does increase to a significant degree the amount of I^{131} collected.

REFERENCES

1. Trunnell, J. B.; Marinelli, L. D.; Duffy, B. J., Jr.; Hill, R.; Peacock, W., and Rawson, R. W.: The treatment of metastatic thyroid cancer with radioactive iodine: credits and debits, *J. Clin. Endocrinol.* **9**: 1138–1152, 1949.
2. Marinelli, L. D.; Foote, F. W., Jr.; Hill, R. F., and Hocker, A. F.: Retention of radioactive iodine in thyroid carcinomas; histopathologic and autoradiographic studies, *Am. J. Roentgenol.* **58**: 17–32, 1947.
3. Bradway, W.: The morphogenesis of the thyroid follicles of the chick, *Anat. Rec.* **42**: 157–167, 1929.
4. Hopkins, M. L.: Development of the thyroid gland in the chick embryo, *J. Morph.* **58**: 585–613, 1935.
5. Coleman, T.: Personal communication from R. W. Rawson, (unpublished).
6. Hansborough, L. A., and Khan, M.: The initial function of the chick thyroid gland with the use of radioiodine (I^{131}), *J. Exper. Zoöl.* **116**: 447–454, 1951.
7. Fugo, N. W.: Effects of hypophysectomy in the chick embryo, *J. Exper. Zoöl.* **85**: 271–291, 1940.

POST-THYROIDECTOMY MYXEDEMA AFTER PREOPERATIVE USE OF ANTITHYROID DRUGS*

ELMER C. BARTELS, M.D.

Department of Internal Medicine, The Lahey Clinic, Boston, Massachusetts

THE development of myxedema in man after complete thyroidectomy was recognized simultaneously by the pioneer in thyroid surgery, Kocher (1), and by Reverdin (2). Since then, the efforts of the thyroid surgeon have been directed toward striking a reasonable balance between removing sufficient thyroid tissue to cure the hyperthyroidism and avoid persistent or recurrent hyperthyroidism, and leaving a remnant of thyroid tissue sufficiently small to prevent postoperative thyroid insufficiency. When such a plan of surgical intervention is followed, various reports have indicated that postoperative myxedema occurs in from 2.9 per cent to as high as 21 per cent of cases (Table 1). The observations of Crile and McCullagh (3) show that the more radical the thyroidectomy, the greater the chance of development of myxedema (myxedema occurred in 4.5 per cent of their cases after conservative thyroidectomy as against 21 per cent of cases after radical thyroidectomy). These observers indicated that the incidence of postoperative hypothyroidism is inversely proportional to the incidence of recurrent hyperthyroidism.

It has been recognized, however, that even after a total thyroidectomy for goiter has been attempted, it is exceptional for profound myxedema to develop. This is contrary to the opinion of Scott and Ramey (9), who advised total thyroidectomy in the management of diffuse toxic goiter and reported that all their patients required supplementary thyroid in doses ranging from $\frac{1}{2}$ to 3 grains (70 per cent required 1 grain and 14 per cent, $1\frac{1}{2}$ grains). That most so-called total thyroidectomies are incomplete and fail to produce a state of athyreosis or hypothyreosis has been proved by the recent reports of Szilagyi (10) and his co-workers and also by Goode, Grollman and Reid (11). By tracer studies with radioactive iodine, Szilagyi found that after a so-called total thyroidectomy, active thyroid tissue was present in the thyroid area in 54.5 per cent of the cases studied and the remaining thyroid fragments were sufficient to maintain the patients

Received for publication May 17, 1952.

* Read at the Annual Meeting of the American Goiter Association, St. Louis, Missouri, May 1–3, 1952.

This article will be included in the bound volume of the 1952 "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1953.

TABLE 1. POSTOPERATIVE (SUBTOTAL THYROIDECTOMY) MYXEDEMA BEFORE USE OF ANTITHYROID DRUGS

Authors	No. of cases reported	Incidence, %	
Gillespie (1930) (4)	209	8.0	
Cattell and Morgan (1939) (5)	4,956	4.6 (6.2 in recurrent disease)	
Grace and Week (1941) (6)	360	2.9	
Hurxthal *et al.* (1945) (7)	643 (Cases prior to 1927; 10-year follow-up)	11.0 Permanent Transient	7.6 3.4
VanderLaan and Swenson (1947) (8)	130	13.9	
Crile and McCullagh (1951) (3)		Prior to 1939—4.5 After 1939—21.0	
		Surgical treatment	
		Conservative	Radical
Graves' disease	162	4.5	21.0
Adenomatous goiter	99	2.0	4.3

in a euthyroid state in 4.4 per cent. By similar technic, Goode, Grollman and Reid found that nontoxic goiters failed to regenerate after total thyroidectomy, but in Graves' disease the thyroid tissue slowly regenerated, so that after one year there was an uptake of radioactive iodine of 25 to 30 per cent and in some cases further treatment with desiccated thyroid was not needed. With these observations showing that the thyroid gland does regenerate, it is surprising that myxedema should be a complication of so-called subtotal thyroidectomy in which all visible thyroid tissue is not removed.

It was thought advisable at this time to review the problem of myxedema since the antithyroid drugs have routinely been employed preoperatively, in order to determine whether there has been any discernible difference in the occurrence of myxedema since their use.

MATERIAL

To date, we have prepared 2,440 hyperthyroid patients for subtotal thyroidectomy by the administration of one of the antithyroid drugs,

in order to bring about an euthyroid state before operation. Of the first 1,000 cases, 942 have been carefully studied by repeated check-up determinations of the basal metabolic rate for periods ranging from three to eight years. This group of 942 cases was carefully studied, so that a review could be made of the problem of postoperative myxedema.

OBSERVATIONS

Development of myxedema. Myxedema developed in 69 of this group of 942 patients, an incidence of 7.3 per cent. In 5.2 per cent the myxedema

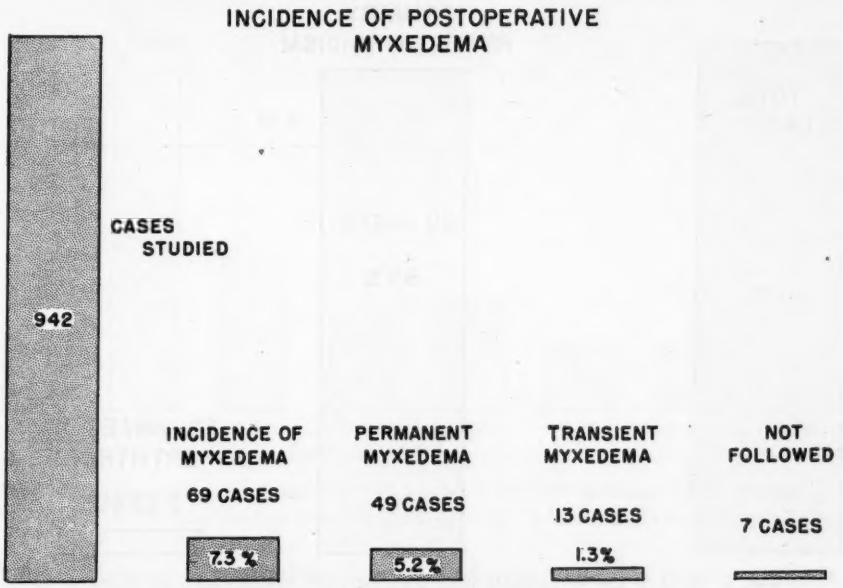


FIG. 1. Postoperative myxedema occurred in 69 of 942 patients, an incidence of 7.3 per cent; it was permanent in 5.2 per cent and transient in 1.3 per cent.

was permanent (Fig. 1). These results compare favorably with those reported by Cattell and Morgan (5) from this clinic before antithyroid drugs were used. There was no sex factor in the development of myxedema; the ratio of women to men was the same as for the entire series (48 women to 21 men). Patients of all ages, ranging from 6 to 68 years, were affected, as is the case in hyperthyroidism.

There was a significant difference in the development of myxedema in patients who had primary hyperthyroidism (diffuse toxic goiter, 66 cases) and in those (3 cases) who had adenomatous goiter with hyperthyroidism (Fig. 2). If, in the entire group of 942 cases, myxedema had developed in patients with adenomatous goiter in the proportion in which it developed

in patients with primary hyperthyroidism, there would have been 15 cases instead of 3, a difference of 80 per cent. That myxedema is more likely to develop in patients who have recurrent primary hyperthyroidism and who require further surgical treatment is shown by the fact that 10 cases with recurrent disease were found in the total of 56 cases. Thus, the likeli-

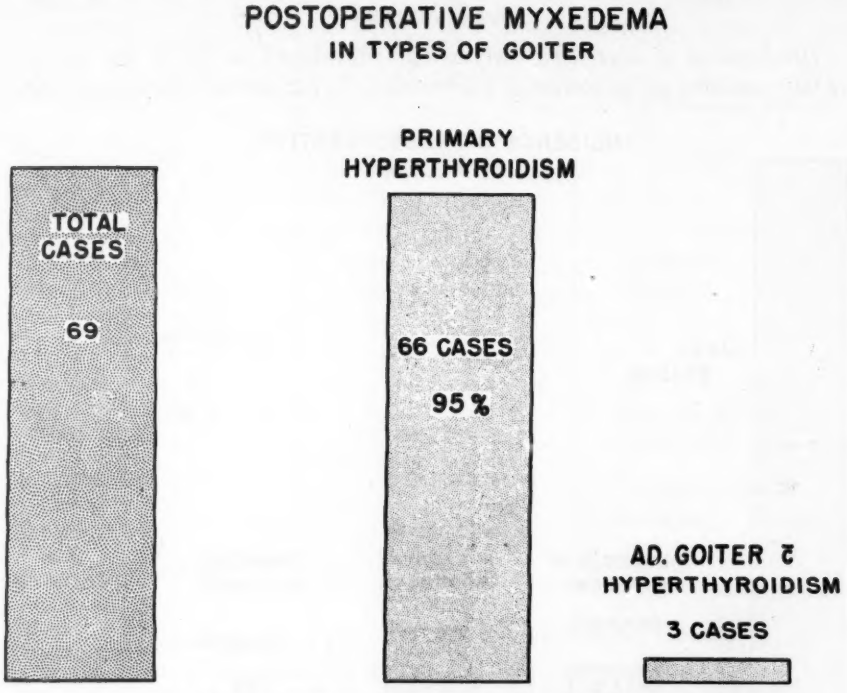


FIG. 2. Most of the 69 patients with postoperative myxedema had primary hyperthyroidism.

hood of the occurrence of myxedema after a second thyroid operation is twice as great (11 per cent) as after the initial operation (6 per cent).

No relationship was apparent between the development of postoperative myxedema and the degree of hyperthyroidism (as determined by the height of the basal metabolic rate or clinical evidence of toxicity), duration of disease, weight of goiter tissue removed or duration of antithyroid treatment. All 69 patients had adequate surgical removal of the thyroid: subtotal thyroidectomy or subtotal removal of bilateral remnants was performed in 66 cases, a unilateral remnant was removed in 2 cases, and a total thyroidectomy was intentionally carried out in only 1 case.

Time of onset of myxedema. The time of onset of myxedema varied wide-

ly, from almost immediately after operation to as late as five years after (Fig. 3). In approximately one half of the cases the diagnosis was made at the time of the first check-up examination, three months after operation. Symptoms were sufficiently troublesome in 2 patients to cause them to return two months after operation. By the end of the first year, the diagnosis of myxedema was made in 87 per cent of the cases in which it occurred. The

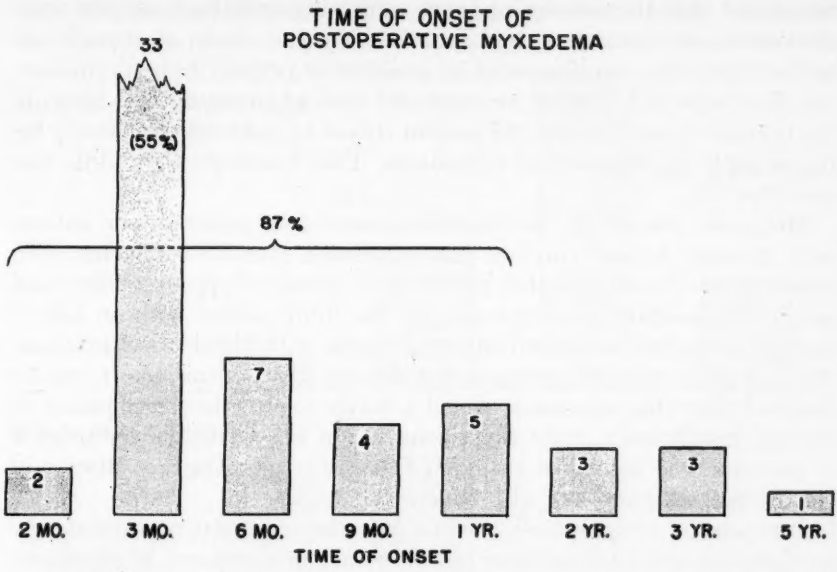


FIG. 3. The onset of postoperative myxedema varied widely, although in 55 per cent it developed by the third month and in 87 per cent by the end of the first year.

longest time to elapse before myxedema became evident was five years. The variability of onset of thyroid insufficiency after operation indicates the unquestionable need for careful periodic check-up examinations of all patients who have had thyroidectomy, particularly those who have had primary hyperthyroidism. This is a responsibility which the surgeon must accept, since myxedema, if left untreated, will lead to unnecessary ill health. Perhaps the poor results that occasionally follow thyroidectomy can be explained on the basis of unrecognized postoperative myxedema. The members of this Association should accept postoperative periodic metabolic check-up examinations as an essential part of the program of treatment. Our observations on patients who have had thyroid surgery performed elsewhere indicate that this is not a routine procedure.

Pathologic study. A study of the pathologic reports revealed two significant findings: 1) strumitis, and 2) malignancy.

Strumitis[1] was associated with primary hyperthyroidism in 12 (17 per cent) of the 69 patients in whom myxedema developed, as compared to only 3 per cent in the entire group of patients who had primary hyperthyroidism—approximately a sixfold increase. Since pathologists do not believe strumitis is a precursor of thyroiditis, a common cause of myxedema, it might be assumed that the strumitis merely replaced active gland tissue and that the amount of tissue remaining contained too few acini for subsequent normal thyroid function. Further study of strumitis is needed before the significance of its presence in primary hyperthyroidism can be interpreted. It must be concluded that when strumitis is found in the thyroid tissue removed, the patient should be examined frequently for the possible development of myxedema. True histologic thyroiditis was not observed.

Malignant changes in the thyroid occurred in 3 patients: one patient with primary hyperthyroidism had associated alveolar carcinoma with blood vessel invasion, another patient with primary hyperthyroidism had associated papillary adenocytoma and the third patient with an adenomatous goiter had associated adenocarcinoma with blood vessel invasion. All 3 patients received roentgen ray therapy after operation. It can be assumed that this treatment played a major role in the development of thyroid insufficiency, since myxedema is not an uncommon occurrence in patients who are given roentgen treatment for malignant disease of the thyroid not associated with hyperthyroidism.

Postoperative complications. Wound infection occurred postoperatively in 2 patients and a tracheotomy was necessary in 2 patients. It is conceivable that these complications could account for the development of destructive septic thyroiditis, which leads to myxedema. However, these complications were so rare that their role as a cause of myxedema is questionable. Postoperative tetany occurred in 5 cases, or 7 per cent. This is much higher than the incidence of 0.7 per cent in unselected cases, indicating that removal of parathyroid tissue or disturbed circulation, the cause of tetany, plays the major role in postoperative myxedema.

Exophthalmos developed in 6 (8 per cent) of the 69 cases. It has been suggested that progressive exophthalmos results from excessive thyroid-stimulating hormone. In these cases it could not be attributed to this stimulation, since in all but 1 case, when the exophthalmos developed the postoperative myxedema was being adequately treated by desiccated thyroid, which is thought to nullify the action of thyroid-stimulating hormone.

Diagnosis of postoperative myxedema. The diagnosis of postoperative

[1] Pathologic interpretation of strumitis: excessive lymphocytic infiltration and fibrosis. Lymphocytic infiltration is always present in the thyroid gland of primary hyperthyroidism (Graves' disease).

myxedema in these cases was determined in one or in a combination of several ways, on the basis of *a*) subjective symptoms, *b*) objective signs, *c*) altered basal metabolic rate, *d*) altered plasma cholesterol, and *e*) altered growth curve in children.

a) Symptoms pointing to thyroid insufficiency were present in 41 of 51 patients about whom information was available. In 10 patients there were no abnormal symptoms. The symptoms most commonly mentioned which suggested myxedema were: intolerance to cold, dryness of the skin,

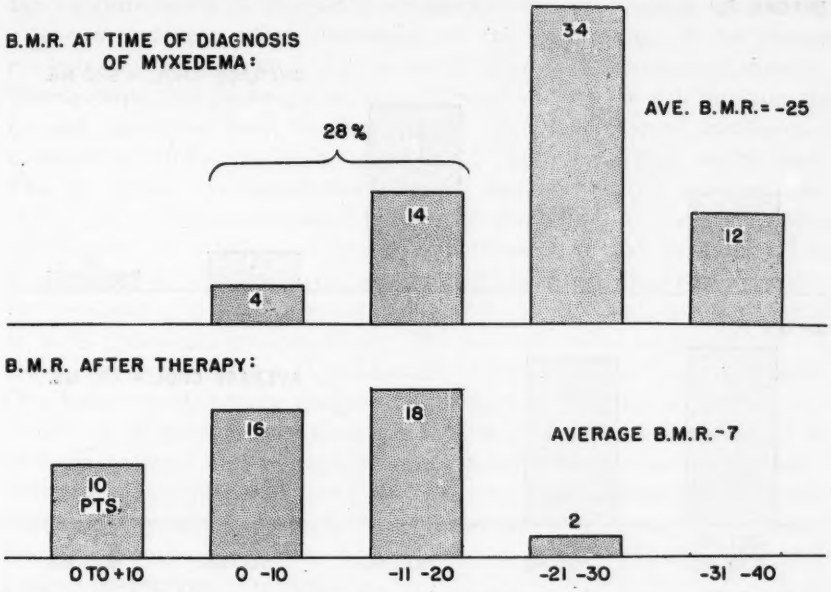


FIG. 4. Twenty-eight per cent of patients with myxedema had basal metabolic rates of ± 0 to -20 per cent (well within the normal range), although the average was -25 per cent. After therapy with desiccated thyroid, the average basal metabolic rate was -7 per cent.

hoarseness, swelling of the eyelids and face, generalized swelling or puffiness of the body and hands, stiffness or cramping of the large muscles, general physical fatigue, increased menstrual flow and mental sluggishness. In no instance were these symptoms marked.

b) The objective signs which suggested myxedema included dryness of the skin, especially over the elbows, hoarseness, lid edema and generalized pallor. Many of the patients in whom these objective signs of myxedema were present did not have subjective symptoms, indicating that the signs of myxedema can precede the symptoms.

c) The basal metabolic rate suggested decreased thyroid function in approximately 70 per cent of cases (Fig. 4). However, since the metabolic

rates were within the accepted range of normal in 30 per cent of the patients, and since it is well recognized that many patients with normal thyroid function have rates as low as −25 per cent, the basal metabolic rate was not always helpful in making a diagnosis of myxedema. The basal metabolic rate was useful as an indication of the responsiveness to

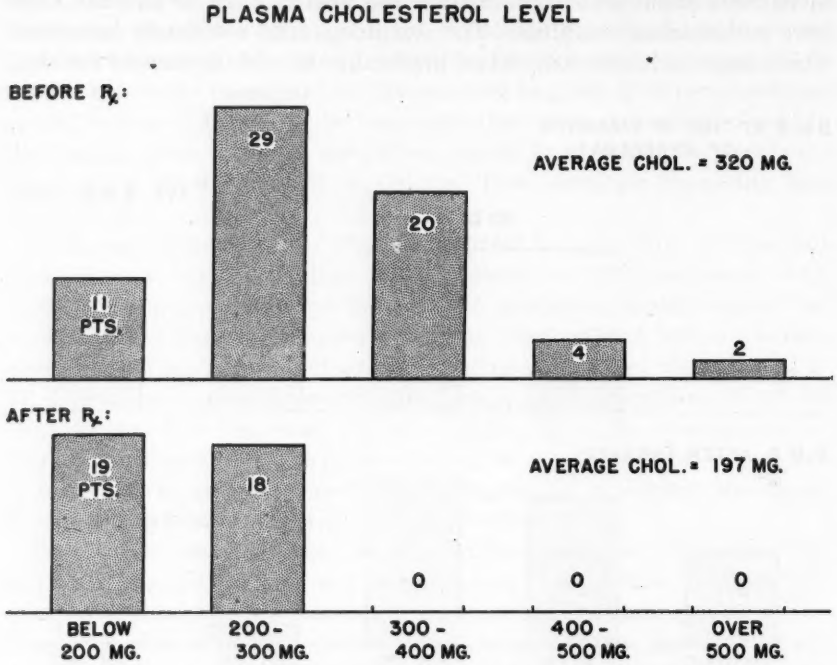


FIG. 5. The average plasma cholesterol level was 320 mg. per 100 cc. in the patients with myxedema; in 11 patients it was less than 200 mg. per 100 cc. After administration of thyroid the average cholesterol level was 197 mg. per 100 cc.

therapy, as shown by the finding that the average basal metabolic rate before treatment was −25 per cent and after therapy, −7 per cent. All basal metabolic rates before treatment were in the minus range; after treatment 10 patients had rates of ±0 or above.

d) The plasma cholesterol level was found to be a most valuable aid in indicating and establishing the diagnosis of myxedema. Some patients in whom a more or less routine plasma cholesterol determination was made, showed values within the myxedematous range, but there was no symptomatic or objective evidence of myxedema. The plasma cholesterol levels ranged from below the 200 mg. level (in 11 patients) to over 500 mg.; in only 6 was the level above 400 mg. (Fig. 5). The average plasma cholesterol value was 320 mg. per 100 cc. After treatment with desiccated thyroid no

cholesterol level was over 300 mg., and the average was 197 mg. It was found that myxedema can be present with an apparently normal cholesterol level, owing to the fact that the normal range of plasma cholesterol is wide. Therefore, even when the cholesterol value was normal in a patient suspected of having myxedema, thyroid treatment led to a substantial fall in the cholesterol level, confirming the diagnosis. (Examples of decreases in cholesterol level: 212 to 146; 265 to 171; 240 to 145, and 266 to 130 mg.) The sharp fall in the concentration of plasma cholesterol following administration of thyroid is evidence of the presence of myxedema.

The experiences which demonstrated the significance of the plasma cholesterol level indicated that it should always be determined preoperatively when the patient is in an euthyroid state. This will indicate the normal cholesterol level for that patient, so that if thyroid insufficiency is suspected after operation, a comparison of cholesterol values can be made. This procedure was carried out in only 8 instances in this series of early cases, but at the present time it is a routine procedure. An elevated plasma cholesterol value following antithyroid therapy should be accepted as evidence of myxedema, even without subjective or objective evidence. On the basis of elevated plasma cholesterol values, 3 patients were found to have myxedema preoperatively. This was before we were aware of the hazards of performing a thyroidectomy while a patient has myxedema. (We have reported these dangers (12), which include intolerance to narcotics and pulmonary complications that may lead to tracheotomy.) One of these patients had postoperative myxedema when seen three months after thyroidectomy; the other 2 patients were euthyroid and subsequently (one and two years, respectively) myxedema developed. This suggests that preoperative myxedema due to excessive antithyroid treatment may lead to postoperative myxedema.

e) Of help in the diagnosis of myxedema in children is the growth curve. This was forcefully demonstrated by a patient who had had a thyroidectomy at the age of 6 years (Fig. 6). This child had hyperthyroidism of eighteen months' duration. When she was first seen her height was 5 inches above normal, a common finding in children who have hyperthyroidism. Propylthiouracil was given for sixty-four days. Preoperatively, the basal metabolic rate was −20 per cent and the plasma cholesterol value was 295 mg., definitely in the myxedematous range. This was one of our very early cases; in spite of the myxedema, a subtotal thyroidectomy was performed and 31 grams of thyroid tissue removed. Because of the myxedema, which led to laryngeal edema, a tracheotomy became necessary. The postoperative course was satisfactory. She was observed by her referring physician who sent periodic metabolic reports; at four months the basal metabolic rate was −10 per cent, at eight months −10 per cent, and at fifteen months −14 per cent. All these rates could be considered

normal. The patient's height fifteen months after operation was 50¾ inches, ¾ inch above the preoperative level. This delay in growth was interpreted as a period of leveling off after the rapid growth during the period of hyperthyroidism. Thirty months after operation the basal metabolic rate was −24 per cent, and the height 51 inches, a growth of only ¼ inch during the previous fifteen months. The referring physician was advised to send the patient for a check-up examination but he failed to do so. Fourteen months later, or forty-four months after operation, the basal metabolic

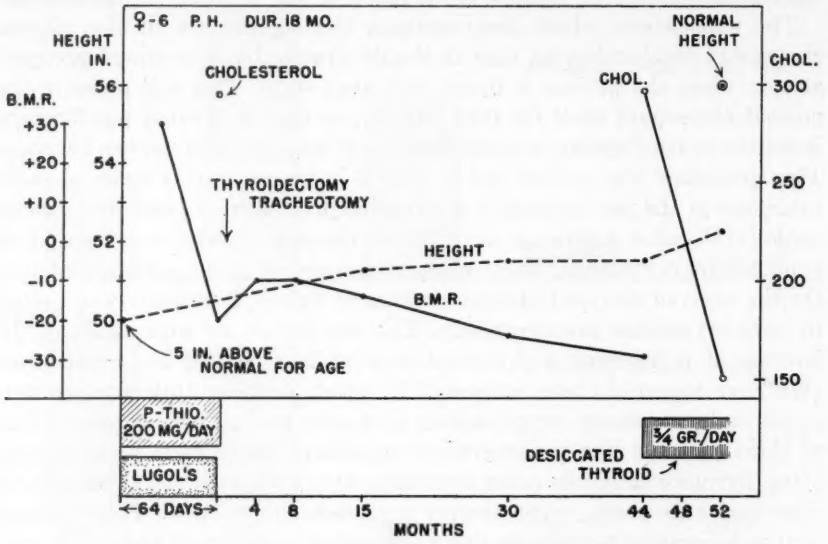


FIG. 6. The lagging growth curve postoperatively in this child led to the diagnosis of postoperative myxedema. Initially the basal metabolic rate was not greatly depressed. The elevated plasma cholesterol level confirmed the diagnosis.

rate was −29 per cent, and the patient's height remained the same. After insistence, she returned for study. Although objectively the patient appeared to be normal and was doing well in school, she had myxedema; the plasma cholesterol level was 298 mg. per 100 cc. After eight months of treatment with ¾ grain of desiccated thyroid daily, the cholesterol level fell to 149 mg. per 100 cc. and there was an increase in height of ¾ inch. At this point, she was 3¾ inches shorter than normal for her age. It is likely that some of this loss in growth will be made up if larger doses of thyroid are given.

Duration of myxedema. Of the entire 69 cases of postoperative myxedema in this series, 13 (19 per cent) were of the transient type. In 10 patients the myxedema lasted less than one year; in 2 other patients thyroid could

be omitted after two years, and in 1 patient, at the end of the third year. The plan for all patients was to omit thyroid medication at least once or twice, to determine whether there was a definite need for continued treatment. A period of six weeks without treatment was advised before check-up examination, to ascertain whether thyroid insufficiency was still present.

Forty-nine patients, or 5.2 per cent, are at present considered to have permanent myxedema. Since 37 of these patients have required thyroid medication for more than three years it is unlikely that they will be able to discontinue therapy. There is, however, a slight chance that therapy can be discontinued in the other 12 who have been treated for only one to two years. Seven patients (8 per cent) were lost to follow-up after the diagnosis of myxedema was established. There was no instance of recurrent hyperthyroidism in the entire series, which indicates that postoperative myxedema, even though transient, is good insurance against recurrent hyperthyroidism.

Treatment. The dose of desiccated thyroid administered to control the myxedema in these cases did not exceed 2 grains a day; 50 per cent of the patients were maintained on 1 grain, 20 per cent on ½ grain, and 20 per cent on 1½ grains a day. The dose of thyroid was governed by determining that amount needed to relieve all signs and symptoms of myxedema and reduce the cholesterol to its lowest possible level. This was accomplished by starting with a small dose of thyroid and gradually increasing the dose until full clinical control was obtained, using the basal metabolic rate and cholesterol determination as guides. The basal metabolic rate alone was not used as a guide for adequate maintenance treatment. Patients were not treated with desiccated thyroid merely because the basal metabolic rate was low, which is so commonly done. Two patients who had angina pectoris were maintained on daily doses of ⅛ grain and ¼ grain, respectively, of desiccated thyroid, and on these small doses they were relieved of all signs and symptoms of myxedema.

SUMMARY

The problem of postoperative myxedema was studied in 942 hyperthyroid patients who received antithyroid drugs before thyroidectomy; antithyroid treatment was carried out until a euthyroid state was attained before operation was performed. Myxedema developed in 69 patients, or 7.3 per cent; in 49, or 5.2 per cent, it was permanent. Postoperative myxedema was twice as common after a second operation.

Myxedema following subtotal thyroidectomy was not found to be any more common after preparation with antithyroid drugs than it was after preparation with iodine.

Most of the patients in whom myxedema developed had primary hyper-

thyroidism. Myxedema rarely occurred in patients with adenomatous goiter.

The pathologic study of the thyroid tissue removed revealed strumitis to be present in a significant number of patients. Further study of the significance of strumitis is needed.

The diagnosis of myxedema in these patients was established on evidence obtained either subjectively and objectively, or by laboratory means (basal metabolic rate and plasma cholesterol level). The plasma cholesterol level was considered most helpful; the basal metabolic rate was less informative. A retarded growth curve is significant in determining the presence of myxedema in children.

Postoperative myxedema was present most frequently by the end of the third month. In some cases the condition did not develop until five years after thyroidectomy, indicating the need for periodic metabolic studies for at least five years after thyroidectomy.

The daily dose of desiccated thyroid required to control postoperative myxedema varied from $\frac{1}{8}$ grain to 2 grains. Myxedema was completely controlled in most cases on daily doses varying from $\frac{1}{2}$ to $1\frac{1}{2}$ grains.

REFERENCES

1. KOCHER: Quoted by Rundle, F. E., *in* Joll: Diseases of the Thyroid Gland, ed.2. London, England, William Heinemann, 1951.
2. REVERDIN: *Ibid.*
3. CRILE, G., JR., and MCCULLAGH, E. P.: The treatment of hyperthyroidism, *Ann. Surg.* **134**: 18–28 (July) 1951.
4. GILLESPIE, M. G.: Postoperative hypothyroidism, *Minnesota Med.* **13**: 235–237 (Apr.) 1930.
5. CATTELL, R. B., and MORGAN, E. S.: Recurrent hyperthyroidism, *Surg., Gynec. & Obst.* **68**: 347–351 (Feb., No. 2A) 1939.
6. GRACE, R. V., and WEEK, C.: Surgery of thyroid in large municipal hospital, *Ann. Surg.* **113**: 496–507 (Apr.) 1941.
7. HURXTHAL, L. M.; SOUDERS, C. R.; DEPERSIO, J. D., and MUSULIN, N.: Ten to twenty year results following subtotal thyroidectomy for primary hyperthyroidism. Preliminary report on 1016 patients operated upon before 1927, *Surg. Clin. North America* **25**: 651–656 (June) 1945.
8. VANDERLAAN, W. P., and SWENSON, O.: Results of surgical treatment in Graves' disease, *New England J. Med.* **236**: 236–238 (Feb. 13) 1947.
9. SCOTT, A. C., JR., and RAMEY, P. M.: Total thyroidectomy in the management of diffuse toxic goiter, *J. Clin. Endocrinol.* **9**: 1048–1053 (Oct.) 1949.
10. SZILAGYI, D. E.; MCCLURE, R. D.; CONNELL, T. H., JR.; WATSON, J. H. L., and PREUSS, L. E.; Radioiodine (I^{131}) tracer studies after total thyroidectomy, *Ann. Surg.* **134**: 546–565 (Oct.) 1951.
11. GOODE, J. V.; GROLLMAN, A., and REID, A. F.: Regeneration of human thyroid after so-called total thyroidectomy, *Ann. Surg.* **134**: 541–545 (Oct.) 1951.
12. LAHEY, F. H., and BARTELS, E. C.: The use of thiouracil, thiobarbital and propylthiouracil in patients with hyperthyroidism, *Ann. Surg.* **125**: 572–581 (May) 1947.

RESULTS OF TREATMENT OF TOXIC GOITER WITH RADIOACTIVE IODINE*

LINDON SEED, M.D. AND BERTHA JAFFÉ, M.D.

From the Department of Surgery, College of Medicine of the University of Illinois, and the Isotope Laboratory, Grant Hospital, Chicago, Illinois

THIS is a report of our results in the treatment of hyperthyroidism with radioactive iodine, plus a review of the clinical results reported in the literature up to the present (April, 1952).

Table 1 contains a complete list of all follow-up studies of a series of patients treated for hyperthyroidism with radioactive iodine. When there have been two or more reports from the same clinic, the latest or most complete is used. It is difficult to arrange different sets of statistics accurately under common headings and the necessary shifting of an investigator's case reports in order to tabulate them in common with those of another investigator creates many errors. These errors are not numerous enough, however, to destroy the validity of the final calculation. No two sets of authors attack the problem of the radiotherapeusis of hyperthyroidism in an identical manner. One group deliberately confines this form of therapy to a small number of patients in whom the risk of surgery is unduly hazardous or to those who have had recurrences following repeated thyroidectomies, whereas another group uses I^{131} in the treatment of practically all patients of all ages, with either toxic diffuse or toxic nodular goiter. Neither is there any uniformity in dosage. One group may give multiple small doses in an effort to avoid too high an incidence of hypothyroidism, whereas another aims as closely as possible at a satisfactory remission with one dose, without much regard for subsequent hypothyroidism. The total of cases treated and analyzed numbers 1,720.

It is probable that four times this number of hyperthyroid patients have actually been treated up to the present. Means (1) has stated that 315 patients have been treated at the Massachusetts General Hospital but only 135 were available for listing in our Table 1; and Yohalem (2) has reported 830 cases at Mt. Sinai Hospital in New York but only 290 could be listed here.

Of the total number of patients, a satisfactory remission was obtained

Received for publication May 22, 1952.

* Read at the Annual Meeting of the American Goiter Association, St. Louis, Missouri, May 1–3, 1952.

This article will be included in the bound volume of the 1952 "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1953.

TABLE 1. SUMMARY OF REPORTS IN THE LITERATURE ON END RESULTS OF TREATMENT OF HYPERTHYROIDISM WITH I^{131}

	Date	No. of patients	Satisfactory remission	Became hypothyroid	Unsatisfactory result	Incomplete; or patient died later
Freedberg, Kurland, Chamomovitz, Ureles (3)	1952	42	25	8	1	8
Scott, Seaman, MacBryde, Gottlieb, Daughaday, Sweeney (4)	1951	195	93	38	1	63
McCullagh, Richards (5)	1951	281	238	19	34	0
Blomfield, Jones, MacGregor, Miller, Wayne (6)	1951	18	16	0	2	0
Horst (7)	1951	25	23	0	2	0
Miller, Dailey, Soley, Foreman, Holmes, Alexander, Sheline (8)	1951	100	79	10	2	9
Moretti (9)	1951	28	22	2	3	0
Schaffner, Friedell, Hummon (10)	1951	18	15	1	0	2
Shipley, Storaasli, Friedell, Potts (11)	1950	47	39	4	4	0
Feitelberg, Kaunitz, Silver, Simon, Wasserman, Yohalem (12)	1950	290	266	18	2	4
Weinbren (13)	1950	11	8	0	3	0
Gordon, Albright (14)	1950	120	109	3	3	5
Moe, Adams, Rule, Moore, Kearns, Clark (15)	1950	100	88	12	0	0
Werner, Goodwin, Quimby, Schmidt (16)	1949	103	85	4	8	6
Williams, Towery, Jaffe (17)	1949	97	84	8	4	1
Kelsey, Haines, Keating (18)	1949	55	36	15	4	0
Chapman, Evans (19)	1949	135	107	8	9	11
Johnson, Ireland, Thomas, Bass (20)	1949	11	9	0	1	1
Prinzmetal, Agress, Bergman, Simkin (21)	1949	44	40	1	3	0
Prinzmetal, Agress, Bergman (22)						
Total		*1720*	*1373*	*151*	*86*	*110*
Per cent		*100*	*80*	*9*	*5*	*6*

in 80 per cent, hypothyroidism of a more or less persistent character occurred in 9 per cent, and the treatment was classified as not satisfactory in 5 per cent. The last column of Table 1 lists those patients who died later of other disease, those who were lost to the follow-up, and those whose treatment was not completed for reasons other than dissatisfaction with the therapy. This group constitutes 6 per cent of the total and could

TABLE 2. SUMMARY OF END RESULTS OF TREATMENT OF HYPERTHYROIDISM WITH I^{131} IN 257 PATIENTS

	No. of patients	Became euthyroid	Became hypothyroid	Improved	No improvement	Died of other disease	Lost to follow-up
Toxic diffuse goiter	135	86	19	12	13	3	2
Recurrent toxic diffuse goiter	67	46	7	6	4	3	1
Toxic nodular goiter	55	31	2	10	7	3	2
Total	*257*	*163*	*28*	*28*	*24*	*9*	*5*
Per cent	*100*	*63*	*11*	*11*	*9*	*3*	*2*

justly be disregarded in the final reckoning. The follow-up of these patients was rather short, since nearly all of them were treated since 1946, at which time I^{131} was made available by the Atomic Energy Commission through Oak Ridge. Although the follow-up is short, the "preview" it affords would indicate that a great many patients with exophthalmic goiter can be cured without surgical intervention.

Table 2 gives a summary of our results in 257 patients followed from six months to two years after treatment with radioiodine. Since the majority of the patients were returned to the care of referring physicians, the status of the patients was determined in most instances by writing or telephoning to their doctors. Thus the evaluation of the end result is based upon the opinions of many doctors and in only a fourth of the cases upon personal examination by the authors. If one includes all three types of goiter, toxic diffuse, recurrent toxic diffuse and toxic nodular, it is seen that a satisfactory remission was obtained in 63 per cent and hypothyroidism occurred in 11 per cent. Improvement was obtained in 28 patients or 11 per cent; 3 of these patients were later operated on. There was no improvement in 24 patients; among these the diagnosis was doubtful in 14. There is probably a greater tendency to treat a patient with questionable hyper-

thyroidism by means of radioiodine than by surgery; and in any series of patients referred for treatment from an ordinary clinical practice there will be a certain number without definite symptomatology, in whom the possibility of improving the symptoms by treatment with radioiodine far outweighs the disadvantages. A few patients in this category obtain improvement; the majority do not. As an indication of the type of patient referred for treatment with I^{131}, one may note the death of 9 patients in a relatively short follow-up period. Of these, 2 patients died of abdominal carcinoma, 6 of organic heart disease in which an important element of hyperthyroidism was hopefully but erroneously suspected, and 1 probably of long continued hyperthyroid heart disease. Five patients were lost to the follow-up. These are the results obtained in an ordinary clinical laboratory functioning largely as a place where physicians in the community may refer their patients for radiotherapeusis and still retain control of the aftercare. As such, the results probably represent the general average that will be obtained throughout the country when radioactive treatment is generally available.

There were some differences observed in the three categories of patients. The patients with toxic diffuse or exophthalmic goiter had an average age of 45 years, and 33 were under the age of 40 years; the average age of the recurrent exophthalmic goiter patients was 49 years, and 8 were under the age of 40; the average age of the toxic nodular goiter patients was 59, and only 3 were under the age of 40 years. A satisfactory remission was obtained in 64 per cent of the patients with exophthalmic goiter, in 68 per cent of those with recurrent exophthalmic goiter, and in 56 per cent of those with toxic nodular goiter; hypothyroidism occurred in 14 per cent, 10 per cent and 4 per cent, respectively.

The normal response to radioiodine therapy consists of a mild degree of radiation thyroiditis during the first week, with real improvement not appearing for three to five weeks. In toxic diffuse cases, the goiter, which has taken on a firmer consistency, begins to diminish in size at that time and practically always disappears if the patient obtains a cure. In toxic nodular cases, the goiter becomes firmer in consistency, is reduced in size, but rarely completely disappears. At six weeks one can estimate by the clinical response the probability of obtaining complete control of the symptoms. McCullagh and Richards (5) found that the time required for remission varied with the size of the goiter; in toxic diffuse goiters under 40 grams of estimated weight the remission time was 3.6 months, but in those of over 70 grams it was 5.1 months, and in toxic nodular goiters 7.6 months. Gordon and Albright (14), by following basal metabolic rates, found that in most of their patients the metabolic rate dropped to a plateau in about three months but between six and nine months there was very often a rebound phenomenon with a rise in metabolism of 10 or 15 per cent, at which

time a mildly hypothyroid individual would usually return to a euthyroid state.

If the damage to the gland is reversible, as these observations would indicate, then successful treatment must be followed by a substantial percentage of relapses. Sufficient time has not elapsed to give a true picture of the recurrence rate. Kelsey and Haines (18) reported 6 recurrences in 51 remissions; McCullagh and Richards (5) estimated their recurrences at 3 per cent; and Shipley and Storaasli (11) had 2 relapses in 20 controlled patients. We have had only 3 patients with toxic diffuse goiter who apparently had a complete remission followed by a recurrence of symptoms, but we believe this number will increase considerably with the passage of time.

Hypothyroidism. The over-all incidence of hypothyroidism in the series collected from the literature is 9 per cent; our incidence is 11 per cent. In the hypothyroid group we have included all patients who are taking desiccated thyroid, no matter how small the dose. The incidence of hypothyroidism is higher with initially higher doses of I^{131} but it actually depends more upon the type of patient that is being treated than it does upon errors in dosage. It would seem that there is a small group of exophthalmic goiter patients whose thyroid glands are quite sensitive to the beta radiation of I^{131} and in whom hypothyroidism promptly develops on doses that would hardly affect most hyperplastic glands and would have no effect on a normal thyroid. In short, a routine initial dose of, let us say, 8 millicuries will produce more myxedematous patients than a dose of 4 millicuries but it will not produce twice as many. Thus, if the clinician is treating only patients who are so ill that they are not considered good surgical risks, he undoubtedly will be recommending a rather high initial dose and consequently will have more hypothyroid sequelae. To be sure, the cautious physician will end up with a lesser number of patients taking desiccated thyroid than will the bolder therapeutist, but the difference in the incidence of hypothyroidism following a regimen of cautious multiple doses and the incidence following a uniform "one shot cure," will not be as great as one would think. In fact, it is exceedingly difficult at times to produce myxedema, even when one wishes to do so.

If hypothyroidism occurs, it most commonly follows the first dose at an interval of two to three months, but in a few instances may not appear for four or five months. A certain level of hypothyroidism is reached in three months, then maintained for another three to six months, following which a rebound phenomenon sets in and not infrequently the patient returns to a euthyroid state. The hypothyroidism following radiotherapeusis is much more likely to be of a transient character than the hypothyroidism following surgery.

We had 9 patients in whom hypothyroidism developed; but they re-

verted to a euthyroid state in six to nine months. One patient became hypothyroid and later became mildly hyperthyroid. The reported percentage of patients with hypothyroidism will depend upon the time interval between treatment and the follow-up study, the incidence being high with a short follow-up and low with a longer follow-up. The highest incidence (25.5 per cent in toxic diffuse goiter) has been reported by Scott and associates (4), a figure which undoubtedly includes a large number of temporarily hypothyroid patients.

Hypothyroidism is less common following treatment for toxic nodular goiter. McCullagh and Richards (5) in treating 78 patients with toxic nodular goiter, had no hypothyroid patients. Scott *et al.* (4), on the other hand, produced hypothyroidism in 8 patients out of 35, but they had only 1 hypothyroid patient among 36 with recurrent toxic diffuse goiters. Gordon and Albright (14) think there is no difference in response to I^{131} therapy between the toxic nodular and toxic diffuse goiters. Among our 55 patients with toxic nodular goiter in only 2 did hypothyroidism develop and in 1 of these the goiter was probably diffusely hyperplastic rather than nodular. The toxic nodular goiter, because of its greater size and lesser ability to pick up iodine, is less amenable to I^{131} therapy; as a result, not only is hypothyroidism less frequent, but so are the cures.

Exacerbation of symptoms. Feitelberg (12) has reported the death of a patient with a 200-gram goiter, apparently in a thyroid storm, twenty-four hours after receiving a therapeutic dose of 10 millicuries of radioactive iodine. An aggravation of the thyrotoxicosis does occur after therapy with I^{131}, but it is not common. In our group 1 patient had a sharp reaction following a 25-millicurie dose; she, strangely enough, had a toxic nodular goiter. Another patient with a recurrence of symptoms following an operation for exophthalmic goiter had a severe reaction after a 10-millicurie dose. Two patients bordering on a thyroid crisis were given 9 millicuries and 10 millicuries, respectively; 2 others, severely toxic with badly decompensated hearts necessitating constant oxygen therapy, were given 15 millicuries apiece; there was no gross clinical evidence of aggravation. At first, because of the fear of producing a dangerous adverse reaction in the very sick patients, we followed the radioactive iodine with Lugol's solution after two or three days, but we have discontinued this maneuver. In the patient who is quite ill and who is pouring out large quantities of the thyroid hormone, the addition of stable iodine occasionally will wash the I^{131} out into the urine, as it is broken off the metabolized thyroxine molecule. This is particularly true if the stable iodine is given only a few days after the I^{131}.

Local reaction. We had only 2 patients who complained of actual pain in the neck and about a dozen who complained of discomfort such as dryness, tightness, difficulty in swallowing, mucus or cough. We did produce a sharp local reaction in a patient who had had a total lobectomy on the left side

for a papillary adenocarcinoma, by administering a 25-millicurie dose in an attempt to destroy the right lobe. Thirty-six hours later this patient experienced such severe pain over the thyroid gland, along the side of the neck, jaw and ear, that it was necessary to give him a hypodermic injection of morphine for relief.

The foregoing observations would indicate an incidence of local reaction of no more than 10 per cent. In checking the literature for reports of this complication we came upon the following statement by Soley, Miller and Foreman (23) "Not infrequently tenderness in the thyroid region was noted within 24 to 72 hours after I^{131} had been given. It was sometimes noted spontaneously by the patient and was nearly always apparent on palpation, especially when 2000 or more microcuries were given in a single dose." After reading this statement we made specific inquiries and examinations in regard to local discomfort, and found that there are very few exophthalmic goiter patients who do not have some evidence of local reaction during the first week following a therapeutic dose of I^{131}. Furthermore, after four to six weeks the number of patients complaining of a sense of constriction in the neck or a lump in the throat or mild difficulty in swallowing, is too great to be attributed, as we first thought, to a purely functional disturbance.

Exophthalmos. The effect of radiotherapeusis on exophthalmos, as far as one can judge, is practically identical to that produced by thyroidectomy. In most instances there is the same cosmetic improvement as after an operation, but actual measurements show little alteration in the proptosis (2, 5, 11, 16, 19, 23).

One of our patients who had only mild eye symptoms before treatment, experienced troublesome exophthalmos and chemosis one month afterward. Heretofore our treatment of patients with progressive or severe exophthalmos has consisted of total thyroidectomy followed by the administration of desiccated thyroid to control the myxedema. Since our results by this method of attack have been satisfactory, we have deliberately administered myxedema-producing doses of I^{131} to patients whose principal disability is due to the exophthalmos. There have been no untoward results so far, but not enough time has elapsed to evaluate the end results. One patient has been definitely improved.

Dosage. There are no satisfactory criteria for estimating dosage. Very few clinicians use the same means of gauging the dose; some laboriously acquire every particle of data that might influence the decision, some inquire not at all into the niceties of transferring a number of millicuries into a specified amount of radiation, some are cautious and quite prudent, some are radical. Yet they all accomplish an amazingly similar therapeutic result.

The following list, summarizing the various methods of determining

dosage, testifies to the wide range of criteria, all of which accomplish practically identical end results:

1. Equivalent roentgens calculated from the thyroid uptake and effective half-life (E. H. L.) of the tracer dose .
2. Equivalent roentgens based upon the uptake of the tracer dose, on the assumption that the E. H. L. is six days.
3. Equivalent roentgens based on the urinary excretion of a tracer dose and the assumption that the remainder of the I^{131} is in the thyroid gland and its E. H. L. is six days.
4. Microcuries per gram retained by the thyroid gland.
5. Microcuries per estimated gram of thyroid.
6. Minimum effective dose, with repetition as indicated.

One does not obtain much information from a calculation of the average total dose. Succeeding doses are not as effective as the first dose, largely

TABLE 3. RESULTS OF INITIAL DOSE OF I^{131} IN TOXIC DIFFUSE GOITER*

Clinical status of patients	Av. dose (mc.)	Retained by thyroid** (mc.)	Av. wt. of gland (Gm.)
Became euthyroid (55 cases)	7.1	5.50	43
Became hypothyroid (14 cases—3 recovered)	8.1	5.26	44
Improved (45 cases)	6.5	4.80	38
No improvement (4 cases)	7.6	5.45	30

* Seventeen cases eliminated from the total number of cases treated (135), because of questionable diagnosis in 11, and incomplete data in 6.

** Estimated on the basis of thyroid uptake of tracer dose.

because of a diminution of thyroidal accumulation and effective half-life (E. H. L.) (3), but perhaps also because of a lessened sensitivity to radiation. The best opportunity to destroy most of the thyroid gland is at the time of the first treatment and it is the result obtained from the initial dose that one is compelled to use as a standard in judging dosage.

Table 3 summarizes the effect of the initial dose in the toxic diffuse goiters. It also lists the average number of millicuries retained by the thyroid gland, assuming (probably erroneously) that it retains the same percentage of the therapeutic dose as it did of the tracer dose. The estimated weight of the goiters is also given, although we do not have much confidence in the accuracy of these hypothetical weights. It can be seen that

there is little correlation between the size of the dose and the result. About all that one could state is that an average initial dose of 7 to 8 millicuries, or of 170 to 180 microcuries per estimated gram of thyroid tissue, or 120 microcuries accumulated per gram of thyroid, given to exophthalmic goiter patients will produce a euthyroid state in about half the cases and hypothyroidism in about 12 per cent. The spread is so great, however, that these averages do not mean much. Hypothyroidism resulted from as little as 3 millicuries in one patient, whereas another patient with a large goiter received a total of 90 millicuries in four doses before control was established.

Table 4 compares the initial dosages when they were followed by either euthyroidism or hypothyroidism, in the three categories, toxic diffuse, recurrent toxic diffuse and toxic nodular goiter. The satisfactory average

TABLE 4. PATIENTS BECOMING EUTHYROID OR HYPOTHYROID AFTER INITIAL DOSE OF I^{131}

Diagnosis	Became euthyroid		Became hypothyroid	
	No. of pts.	Av. dose (mc.)	No. of pts.	Av. dose (mc.)
Toxic diffuse goiter (135 pts. treated)	55	7.1	14	8.1
Recurrent toxic diffuse goiter (67 pts. treated)	23	8.9	7	9.3
Toxic nodular goiter (55 pts. treated)	21	13.3	1	5.0

initial dose is slightly higher in recurrent toxic diffuse goiter (8.9 mc.) than in intact toxic diffuse goiter (7.1 mc.) and nearly twice as high in toxic nodular goiter (13.3 mc.). This difference is explained in part by the fact that the thyroid pickup in toxic diffuse goiter as determined by the tracer study was 71 per cent, in recurrent diffuse goiter it was 62 per cent and in toxic nodular goiter only 47 per cent. If one calculates the number of millicuries theoretically retained by the thyroid glands in these groups, it then comes to only 5, 5.5 and 6 millicuries, respectively. The average estimated weight of the toxic nodular goiters was 70 grams, so the dose retained per gram of thyroid was very much smaller than in diffuse goiter. Radioautographic studies have shown a more spotty distribution of I^{131} in nodular goiters (24); this may also interfere with a satisfactory destruction of the gland by internal ionizing radiation. We have increased our doses during the year and have administered an average initial dose of 8.6 mc. in toxic diffuse goiter, 9.4 mc. in recurrent diffuse goiter and 16.2 mc. in toxic

nodular goiter. Under this regimen, in the patients with diffuse goiter, 50 to 60 per cent will obtain a remission after the first dose and 15 per cent will become hypothyroid (most of whom recover). Of the toxic nodular goiter patients, 40 per cent will obtain a remission and very few will become hypothyroid. We do not approve generally of treating toxic nodular goiter with I^{131} and refuse to do so if the gland is large and the thyroid uptake of the tracer dose is below 15 per cent. Patients with toxic nodular goiter accepted for treatment are usually in such poor condition that immediate and certain improvement is mandatory, so we very often administer an initial dose of 25 mc.

Theoretically one should be able to predict the proper dose most accurately by estimating the weight of the gland, determining the uptake and effective half-life of the tracer dose, and then calculating the dosage in equivalent reontgens by the simple formula of Marinelli and Quimby (25). This procedure requires much time and labor and it is doubtful whether there is sufficient increased dependability of results to justify the extra work. Miller and Sheline (26) of the University of California have lost faith in the method, but Freedberg and his colleagues (3, 27, 28) have recently presented an excellent analysis indicating that the procedure does have value. It is our opinion that in a frank exophthalmic goiter of moderate to severe toxicity the uptake and the E.H.L. of the treatment dose is on the whole considerably less than that of the tracer dose and that calculations based upon an assumed duplication of tracer and treatment doses are frequently erroneous, not only as a result of technical errors inherent in the methods of measurement, but also as a result of metabolic alterations. Of 53 patients upon whom we made a comparison between the predicted and delivered dose, we found that in only 16 patients the therapeutic dose in roentgens fell within a plus or minus 15 per cent deviation from the predicted dose, in 2 patients it was above 15 per cent, and in 35 patients it varied from 45 to 85 per cent below the predicted dose.

From a scientific point of view the determination of the dose of I^{131} to be administered to a hyperthyroid patient will depend upon 1) the percentage of I^{131} picked up by the thyroid gland as determined by the tracer study, 2) the effective life of the radioactivity in the gland, 3) the size of the gland, and 4) the type of goiter. From a practical point of view the dose will depend upon 1) the desire to control quickly and certainly the symptoms, regardless of a subsequent high incidence of hypothyroidism, or 2) the desire to reduce the hyperthyroidism to euthyroidism without causing hypothyroidism, regardless of the number of doses or the amount of time such perfection will require. Thus, the factors involved in determining the number of millicuries to be given to a particular patient will be about half theoretical and half practical. Probably as good a rule to follow as any, is that

proposed by McCullagh and his associates (5) and by Williams and his associates (29). They begin with a 4-mc. dose to an individual with a normal sized gland and add 1 mc. for each additional 10 grams of gland. Usually the more toxic the patient the larger is his goiter, so it is difficult to present statistics proving that the greater the toxicity the greater must be the dose; but this is certainly our clinical impression as well as that of others. In many patients with recurrent exophthalmic goiter there is no palpable thyroid—nothing but a scar on the neck. It is impossible to regulate dosage based upon microcuries per gram of thyroid. One might assume that because there is no palpable goiter a small dose would be sufficient. This has not been our experience. The patient with recurrent symptoms of moderate to severe degree and no palpable gland requires 6 to 8 millicuries for effective treatment. A great many side factors of a practical nature will influence the choice of the initial dose, all of them quite obvious to the clinician when he is presented with a particular patient. It is wise to give the minimum effective dose in a young patient without much thyroid enlargement, add a few millicuries in an elderly patient, add a few more if the symptoms are severe, double the dose if the heart is badly damaged, subtract a millicurie or two if the patient is already overweight, and add a couple if the patient lives out of town or if the patient's doctor is dubious of the value of the treatment. In toxic nodular goiter, under the usual circumstances, one may as well start I^{131} therapy with 20 millicuries and add 10 more if there is a large goiter.

SUMMARY AND CONCLUSIONS

1. In a series of 257 hyperthyroid patients followed from six months to two years after treatment with I^{131}, a satisfactory remission was obtained in 63 per cent, hypothyroidism occurred in 11 per cent and in 25 per cent the results were considered to be unsatisfactory.

2. In 1,720 similar cases reported in the literature up to the present, a remission was obtained in 80 per cent, hypothyroidism occurred in 9 per cent and in 11 per cent the results were classified as incomplete or unsatisfactory.

3. Complications were very few and none was serious. Genetic injury and carcinogenic effect remain purely hypothetical possibilities.

4. The administration of I^{131} is the treatment of choice in recurrent toxic diffuse goiter and in most toxic diffuse goiters in patients over 45 years of age. Patients with large goiters are more difficult to cure and it is probably better to operate upon them. With this exception, it may be that all exophthalmic goiter patients are best treated by radioiodine.

5. Surgery is the treatment of choice in toxic nodular goiter, except under unusual circumstances. The operation is a very satisfactory procedure,

cure is certain, the goiter is removed, and complications and recurrences are rare. Cure by I^{131} is more difficult and less certain. In addition, the goiter itself rarely entirely disappears.

6. There are no criteria by which one can accurately estimate dosage of I^{131}. A tracer dose is necessary to determine the presence and degree of thyroid uptake. In toxic diffuse goiter the uptake is usually in the neighborhood of 70 per cent. Under these circumstances, the average patient with a small goiter can be given 6 to 8 millicuries as an initial dose. The dose may be decreased to 4 millicuries if the symptoms are mild and if the goiter is very small, or it may be increased up to 15 or 20 millicuries if the goiter is very large and the symptoms severe. Under this regimen, control of symptoms is obtained in over half the cases after the initial dose, and hypothyroidism appears in another 10 to 15 per cent.

7. In toxic nodular goiter the thyroid uptake is lower. If it is below 20 per cent, treatment with I^{131} is not advisable under almost any circumstances. The initial dose in a patient with a toxic nodular goiter of moderate size is 20 millicuries; if the goiter is large or the symptoms severe, this can be raised to 35 millicuries. Repeated doses at intervals of two months are usually necessary. The goiter becomes smaller but does not entirely disappear, even when the hyperthyroidism is eliminated.

REFERENCES

1. Means, J. H.: The use of hormones, drugs, and radiations in the management of thyroid disease, *Bull. Johns Hopkins Hosp.* **89**: 106–120 (Aug.) 1951.
2. Yohalem, S. B.: Treatment of hyperthyroidism with radioiodine, *New York Med.* **7**: 20–22 (Oct.) 1951.
3. Freedberg, A. S.; Kurland, G. S.; Chamovitz, D. L., and Ureles, A. L.: A critical analysis of the quantitative I^{131} therapy of thyrotoxicosis, *J. Clin. Endocrinol. & Metab.* **12**: 86–111 (Jan.) 1952.
4. Scott, W. G.; Seaman, W. B.; MacBryde, C.; Gottlieb, L.; Daughaday, W. H., and Sweeney, B. J.: Observations and results in treatment of hyperthyroidism with radioactive iodine (I-131), *Am. J. Roentgenol.* **66**: 171–178 (Aug.) 1951.
5. McCullagh, E. P., and Richards, C. E.: Radioactive iodine in treatment of hyperthyroidism, *Arch. Int. Med.* **87**: 4–16 (Jan.) 1951.
6. Blomfield, G. W.; Jones, J. C.; MacGregor, A. G.; Miller, H., and Wayne, E. J.; Treatment of thyrotoxicosis with radioactive iodine, *Brit. M. J.* **2**: 373–381 (Aug.) 1951.
7. Horst, W.: Radiotherapy of thyroid hyperfunction with radioactive iodine, *Deutsch. med. Wchnschr.* **76**: 1052-1056 (Aug.) 1951.
8. Miller, E. R.; Dailey, M. E.; Soley, M. H.; Foreman, H.; Holmes, A.U. Alexander, G. L., and Sheline, G. E.: Studies with radioiodine. II. Treatment of patients with hyperthyroidism by I-131, *Radiology*, **57**: 227–233 (Aug.) 1951.
9. Moretti, E.: Radioactive iodine in diagnosis and therapy of hyperthyroidism, *Policlinico* (Sez. prat.) **58**: 867–872 (July 9) 1951.
10. Schaffner, F.; Friedell, M. T., and Hummon, I. F.: Radioiodine (I-131) in the

diagnosis and treatment of diseases of the thyroid, *Illinois M. J.* **99**: 15–21 (Jan.) 1951.

11. Shipley, R. A.; Storaasli, J. P.; Friedell, H. L., and Potts, A. M.: I^{131} in the diagnosis and treatment of hyperthyroidism, *Am. J. Roentgenol.* **64**: 576–589 (Oct.) 1950.
12. Feitelberg, S.; Kaunitz, P. S.; Silver, S.; Simon, N.; Wasserman, L. S., and Yohalem, S. B.: Hyperthyroidism; treatment with radioactive iodine, *Arch. Int. Med.* **85**: 471–478 (March) 1950.
13. Weinbren, M.: Radioactive iodine in the treatment of thyroid diseases: a review of the literature and preliminary report of cases treated, *South African J. Clin. Sc.* 213–289 (Sept.) 1950.
14. Gordon, E. S., and Albright, E. C.: Treatment of thyrotoxicosis with radioactive iodine, *J.A.M.A.* **143**: 1129–1132 (July 29) 1950.
15. Moe, R. H.; Adams, E. E.; Rule, J. H.; Moore, M. C.; Kearns, J. E., Jr., and Clark, D. E.: An evaluation of radioactive iodine in the treatment of hyperthyroidism, *J. Clin. Endocrinol.* **10**: 1022–1028 (Sept.) 1950.
16. Werner, S. C.; Goodwin, L. D.; Quimby, E. H., and Schmidt, C.: Some results from the use of radioactive iodine, I^{131}, in the diagnosis and treatment of toxic goiter, *Am. J. Roentgenol.* **63**: 889–894 (June) 1950.
17. Williams, R. H.; Towery, B. T., and Jaffe, H.: Radioiodotherapeusis, *Am. J. Med.* **7**: 702–717 (Dec.) 1949.
18. Kelsey, M. P.; Haines, S. F., and Keating, F. R.: The treatment of exophthalmic goiter with radioiodine, *Postgrad. Med.* **6**: 263–273 (Oct.) 1949.
19. Chapman, E. M., and Evans, R. D.: Treatment of toxic goiters with radioactive iodine, *Med. Clinics North Am.* **33**: 1211–1223 (Sept.) 1949.
20. Johnson, J. B.; Ireland, C.; Thomas, R. F., and Bass, A.: Treatment of hyperthyroidism with radioactive iodine, *Am. J. Med.* **6**: 504–505 (Apr.) 1949.
21. Prinzmetal, M.; Agress, C. M.; Bergman, H. C., and Simkin, B.: Problem cases of toxic diffuse goiter treated with radioactive iodine, *J.A.M.A.* **140**: 1082–1089 (July 30) 1949.
22. Prinzmetal, M.; Agress, C. M., and Bergman, H. C.: The use of radioactive iodine in the treatment of Graves' disease, *Calif. Med.* **64**: 236–239 (Apr.) 1949.
23. Soley, M. H.; Miller, E. R., and Foreman, N.: Graves' disease: treatment with radioiodine (I^{131}), *J. Clin. Endocrinol.* **9**: 29–35 (Jan.) 1949.
24. Dobyns, B. M., and Lennon, B.: A study of histopathology and physiologic function of thyroid tumors, using radioactive iodine and radioautography, *J. Clin. Endocrinol.* **8**: 732–748 (Sept.) 1948.
25. Marinelli, L. D.; Quimby, E. H., and Hine, G. J.: Dosage determination with radioactive isotopes, *Am. J. Roentgenol.* **59**: 260–281 (Feb.) 1948.
26. Miller, E. R., and Sheline, G. E.: Studies with radioiodine: problem of dosage in the treatment of hyperthyroidism, *Radiology* **57**: 720–728 (Nov.) 1951.
27. Freedberg, A. S.; Chamovitz, D. L., and Kurland, G. S.: Thyroid function in normal and pathological states as revealed by radioactive iodine studies. I. Thyroid I^{131} uptake and turnover, *Metabolism* **1**: 26–35 (Jan.) 1952.
28. Freedberg, A. S.; Chamovitz, D. L., and Kurland, G. S.: *Id.* II. Factors influencing the uptake and turnover of I^{131} by the thyroid gland, *Ibid.*, pp. 36–48.
29. Williams, R. H.; Jaffe, H.; Towery, B. T.; Rogers, W. F., and Tagnon, R.: Factors influencing the effectiveness of radioiodotherapeusis, *Am. J. Med.* **7**: 718–730 (Dec.) 1949.

Editorial

JOURNAL OF CLINICAL ENDOCRINOLOGY

Publications Committee: Warren O. Nelson (Chairman), Edward W. Dempsey, Roy O. Greep, Roy G. Hoskins, E. Perry McCullagh, Willard O. Thompson.

Editorial Board: Willard M. Allen, R. M. Archibald, C. L. Buxton, Robert A. Cleghorn, Frank L. Engel, Ray F. Farquharson, Thomas F. Gallagher, Samuel F. Haines, Edwin C. Hamblen, Norris J. Heckel, Roy G. Hoskins, John E. Howard, Laurance W. Kinsell, Harold L. Mason, E. Perry McCullagh, Warren O. Nelson, Leo T. Samuels, Randall G. Sprague, George W. Thorn, Lawson Wilkins.

Managing Editor: Willard O. Thompson.

ACTH AND CORTISONE—AFTER NEARLY FOUR YEARS OF INTENSIVE CLINICAL AND EXPERIMENTAL EVALUATION

AS OF the autumn of 1949, there was an impression in some quarters that corticotropin and cortisone, singly or together, represented the long sought philosophers' stone. Three years later, some of those who had been most sure that the hormones would cure everything, were equally vehement in stating that they were not only dangerous but worthless.

Early evaluation of the effects of corticotropin and cortisone made it apparent to the clinical physiologist that whatever clinical value the agents might have, they *cured nothing*, and they exerted their rather dramatic effects in various disease states by interposing some sort of a blockade between the noxious agents involved and the tissue cells of the afflicted individual. In the case of such diseases as rheumatoid arthritis and bronchial asthma, essentially diseases of unknown etiology, the interposition of such a blockade could result in complete rehabilitation of the patient. If the story had ended there, the picture would have been relatively simple. This, however, was not the case. As every student of Cushing's syndrome was aware, cortisone-like steroids are capable of producing abnormalities characterized, among other things, by wasting of protein tissue, retention of salt and water, and a tendency to abnormal carbohydrate metabolism. It was perhaps the belated recognition of this unpleasant fact which caused some of the early enthusiasts to manifest a delayed alarm reaction of no small magnitude.

No one is as yet equipped to define the precise place of ACTH and cortisone in clinical medicine. On the basis of some years of experimental evaluation and nearly four years of clinical evaluation, however, certain definite statements are permissible:

1. An impressive number of individuals are alive and essentially well, who without ACTH and/or cortisone almost certainly long since would have died. Prominent among this group are patients with pemphigus, dermatomyositis, lupus erythematosus, and other manifestations of the so called "collagen diseases." We believe also that the same statement may be made in regard to a significant number of individuals with specific infectious diseases, in whom well indicated antibiotic therapy alone was insufficient to produce clinical improvement and in whom a brief period of combined hormonal and antibiotic therapy resulted in a "tiding over" of the patient until the specific antibiotic agent had time to do its work.

2. Since the advent of ACTH and cortisone, many patients with chronic disabling diseases who had previously been completely unable to pursue a gainful occupation, have become self-supporting, contributing members of society. Patients with severe rheumatoid arthritis and bronchial asthma are in the forefront of this group.

3. Serious difficulties have been encountered in the management of many patients during short-term and long-term hormonal therapy of this type, as had been anticipated by those who had worked in the pituitary-adrenal field prior to the "clinical era." The difficulties have included all those things which are found in spontaneously occurring Cushing's syndrome. In addition, the occasional occurrence of psychotic manifestations, and of perforation of peptic ulcer represent major hazards.

4. Proper attention to "anti-ACTH-cortisone measures" prevents or minimizes most of the untoward effects. Evaluation of such measures has been one of the major undertakings in this laboratory and clinic during the past two years. The use of diets high in protein (nucleoprotein in particular), high in potassium, low in sodium, low in carbohydrate, adequate in calories and adequate in essential vitamins, together with the use of anabolic steroids such as testosterone and estrogen, enables many individuals to receive continuous "blockade therapy" for periods of months and years without evidence of clinical or obvious biochemical abnormalities. Deviation from such a dietary program in these patients (without any change in hormonal therapy) frequently results in rapid appearance of manifestations resembling those of Cushing's syndrome.

5. In the chronic non-fatal disabling disease states in which ACTH and cortisone are effective, either of two types of dosage may be employed. One procedure is to administer sufficient to make a patient comfortable without completely eliminating signs and symptoms of the disease. In so doing, the hormones are employed merely as "superaspirins." The other procedure is to use amounts of the hormones sufficient to suppress the disease picture completely. Initially, this requires high dosage, and correspondingly increases the likelihood of untoward manifestations. On the

basis of experience in this laboratory and clinic, however, the net long-term results obtained by such a procedure are vastly to be preferred to those obtained by the "superaspirin" policy.

It is to be hoped that the periods of extreme euphoria and of extreme alarm in the field of pituitary-adrenal therapy are behind us. If one surveys the subject soberly, it is apparent that ACTH and cortisone have a most important place in clinical medicine; that their use involves a significant amount of hazard; that, with the knowledge at present available, many of these hazards can be avoided; and that a vast amount of work remains to be performed in terms of clinical and laboratory evaluation.

L. W. KINSELL

Letters to the Editor

RELATIVE INSENSITIVITY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL SYSTEM TO ACTIVATION BY EPINEPHRINE

To the Editor:

The case of coexisting pheochromocytoma and adrenal cortical adenoma presented by Cope *et al.* in this journal (1) brings up some points for discussion which seem particularly important at this time.

The hypothesis that the increase in *l*-epinephrine secretion by the pheochromocytoma may have led to the production of the cortical adenoma is certainly attractive and seemingly quite likely. It may be necessary, as Dr. Cope also points out, to have a high concentration of *l*-epinephrine in the pheochromocytoma and a more or less continuous secretion from the tumor to induce formation of an adenoma. This seems to offer the best explanation as to why this combination of tumors has not been observed in the past, and it falls in line with available clinical evidence which strongly suggests that epinephrine is at best a poor stimulant of the hypothalamic-pituitary system for ACTH release, except under special conditions.

There is little doubt that epinephrine will cause ACTH release from the pituitary, and the experiments of Vogt (2), Long (3), Hume and Wittenstein (4) and others have shown this. The relative insensitivity of the hypothalamic-pituitary system to activation by epinephrine is apparently not too well appreciated and deserves comment.

Jeffries *et al.* (5) have recently shown that ordinary clinical dosages of epinephrine (0.3 mg.) will not cause any significant change in potassium or 17-ketosteroid excretion in normal adults, even though circulating eosinophils are depressed. Also, the four-hour epinephrine test, often erroneously referred to as the Thorn test, has not proven to be dependable in screening adrenal cortical insufficiency. Knowlton (6) has already recommended that this test be discarded, for the simple reason that patients with known Addison's disease (not receiving cortisone therapy) may show a better than 50 per cent drop in eosinophils four hours after a subcutaneous dose of 0.3 mg. of epinephrine. Finally, Nelson *et al.* (7) have been unable to demonstrate any increase in the blood levels of 17-hydroxycorticosteroids following four-hour intravenous infusions of epinephrine (1 to 3 mg.) into normal men, even though the eosinophils were markedly depressed.

From the foregoing data, it is clear that epinephrine in the dosages employed does little more than produce an eosinopenia, and even this may occur in the presence of known Addison's disease. Although small doses of epinephrine release small amounts of ACTH, there is evidence that eosinopenia after epinephrine may result from peripheral synergism with the eosinopenic action of 11-17-oxysteroids such as cortisone (8). When eosinopenia follows administration of epinephrine, there is no way in which the contribution of this factor can be assessed.

Epinephrine under special conditions will cause considerable amounts of ACTH to be released from the pituitary. Forsham *et al.* (9) have described a case of Cushing's syndrome in which 0.3 mg. of epinephrine given every six hours for one day doubled the output of 17-ketosteroids. In addition, Wolfson (10) has shown that when large amounts of epinephrine (16–32 mg.) are given daily in conjunction with a sympatholytic dihydrogenated ergot alkaloid, a marked increase in 17-ketosteroid output occurs. Clinical remissions of acute optic neuritis and atopic eczema occurred concomitantly in his patients. These observations suggest that adequate release of ACTH occurs (a) if the hypothalamic-pituitary system is hyperactive, or (b) if excessively large unphysiologic doses of epinephrine are employed.

Present clinical evidence seems, then, to warrant the following conclusions:

1. Epinephrine is quantitatively a poor stimulant of the hypothalamic-pituitary-adrenal system.
2. The four-hour epinephrine test is not a valid test to employ in screening patients for adrenal cortical insufficiency, and should be discarded.
3. Although there is little doubt that epinephrine will cause pituitary ACTH release, its use for this purpose is not practical or dependable.

RICHARD J. MEYER, M.D.

Medical Department,
The Armour Laboratories,
520 North Michigan Avenue,
Chicago 11, Illinois

REFERENCES

1. COPE, O.; LABBE, J. P.; RAKER, J. W., and BLAND, E. F.: Pheochromocytoma and adrenal cortical adenoma. Report of a case with both tumors and discussion of their relation, *J. Clin. Endocrinol. & Metab.* **12**: 875, 1952.
2. VOGT, M.: Observations on some conditions affecting rate of hormone output by suprarenal cortex, *J. Physiol.* **103**: 317, 1944.
3. LONG, C. N. H.: Conditions associated with secretion of adrenal cortex, *Fed. Proc*, **6**: 461, 1947.

4. HUME, D. M., and WITTENSTEIN, G. J.: The relationship of the hypothalamus to pituitary-adrenocortical function, *in* Proceedings of the First Clinical ACTH Conference (Chicago, 1949), edited by J. R. Mote. Philadelphia, The Blakiston Company, 1950, p. 134.
5. JEFFRIES, W. MCK.; BOCHNER, A. K., and DORFMAN, R. I.: A comparison of effects of single injections of ACTH and epinephrine upon adrenal cortical activity, (abstract) *J. Clin. Endocrinol. & Metab.* **12**: 924, 1952.
6. KNOWLTON, A. I.: The modern treatment of Addison's disease, *Med. Clin. North America* **36**: 721, 1952.
7. NELSON, D. H.; SANDBERG, A. A.; PALMER, J. G., and GLENN, E. M.: Levels of 17-hydroxycorticosteroids following intravenous infusion of epinephrine into normal men, (abstract) *J. Clin. Endocrinol. & Metab.* **12**: 936, 1952.
8. THORN, G. W.; FORSHAM, P. H.; FRAWLEY, T. F.; WILSON, D. L.; RENOLD, A. E.; FREDRICKSON, D. S., and JENKINS, D.: Advances in the diagnosis and treatment of adrenal insufficiency, *Am. J. Med.* **10**: 595. 1951.
9. FORSHAM, P. H.; THORN, G. W.; FRAWLEY, T. F., and WILSON, D. L.: Studies on carbohydrate metabolism in adrenal cortical hypersecretion, (abstract) *J. Clin. Endocrinol.* **10**: 825, 1950.
10. WOLFSON, W. Q.: Inadequacy of epinephrine as an activator of the pituitary-adrenal system, (Letter to the Editor) *J. Clin. Endocrinol. & Metab.* **13**: 125, 1953.

INADEQUACY OF EPINEPHRINE AS AN ACTIVATOR OF THE PITUITARY-ADRENAL SYSTEM

TO THE EDITOR:

Particularly in man, the ability of *l*-epinephrine to induce a significant increase in ACTH secretion remains uncertain. As Dr. Richard Meyer has indicated (1), this stems partly from growing dissatisfaction with the 0.3–mg. epinephrine test and partly from failure to obtain evidence of distinct increases in adrenal function when subjects were given larger total daily doses of adrenalin in divided amounts.

At the time the experiments to be described were undertaken, it seemed likely that *l*-epinephrine could release some ACTH from the human pituitary but that the amount of ACTH liberated was small when released by any well-tolerated dose of *l*-epinephrine. It seemed advisable to investigate the possibility that larger total dosages of *l*-epinephrine might be required for release of important amounts of ACTH. This was accomplished by the simultaneous administration of *l*-epinephrine and a sympatholytic mixture of dihydrogenated ergot alkaloids (CCK-179, Sandoz). The choice of CCK-179 was based upon low intrinsic toxicity and upon unpublished experiments in which the Teppermans had shown that rather large doses of ergotamine tartrate did not cause depletion of adrenal ascorbic acid in the intact rat and also did not interfere with the depletion of adrenal ascorbic acid produced in the same animals by the intraperitoneal administration of a known alarming agent (2). It thus appeared likely that the

sympatholytic ergot alkaloids were not potent alarming agents themselves (in contrast to some other sympatholytic agents such as dibenamine) and that they did not block the hypothalamo-pituitary-adrenal response to systemic stress. In addition, in preliminary human studies, administration of 1 or 2 mg. of CCK-179 with 0.3 mg. of *l*-epinephrine did not modify the eosinopenic response to the latter (3).

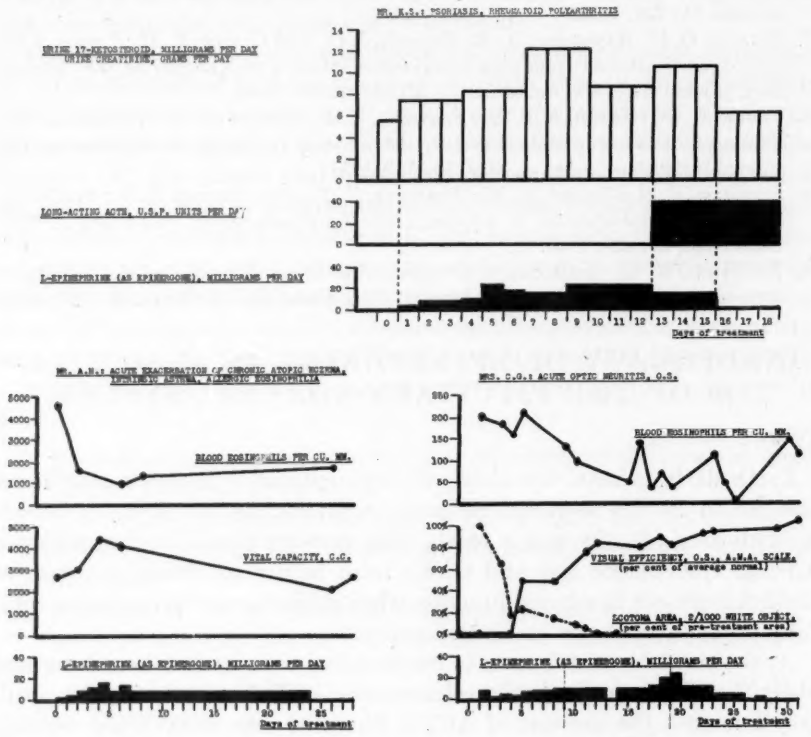

MR. K.S.: PSORIASIS, RHEUMATOID POLYARTHRITIS
URINE 17-KETOSTEROID, MILLIGRAMS PER DAY
URINE CREATININE, GRAMS PER DAY
LONG-ACTING ACTH, U.S.P. UNITS PER DAY
L-EPINEPHRINE (AS EPINERGONE), MILLIGRAMS PER DAY
Days of treatment
MR. A.N.: ACUTE EXACERBATION OF CHRONIC ATOPIC ECZEMA, PERENNIAL ASTHMA, ALLERGIC EOSINOPHILIA
BLOOD EOSINOPHILS PER CU. MM.
VITAL CAPACITY, C.C.
L-EPINEPHRINE (AS EPINERGONE), MILLIGRAMS PER DAY
Days of treatment
MRS. C.G.: ACUTE OPTIC NEURITIS, O.S.
BLOOD EOSINOPHILS PER CU. MM.
VISUAL EFFICIENCY, O.S., A.M.A. SCALE (per cent of average normal)
SCOTOMA AREA, S/1000 WHITE OBJECT (per cent of pre-treatment area)
L-EPINEPHRINE (AS EPINERGONE), MILLIGRAMS PER DAY
Days of treatment

FIGURE 1

The mixture of *l*-epinephrine and CCK-179 used in the definitive human studies contained 2 mg. each of hormone and of blocking agent per ml. of long-acting base, and was designated Epinergone. When dosage was gradually increased over a period of days, most subjects tolerated a daily dose of 16 to 32 mg. of *l*-epinephrine (4 to 8 mg. every six-hours) given as Epinergone. One factor which limited dosage was the difficulty of obtaining an exactly simultaneous onset of action of both agents; thus some subjects had signs of unneutralized epinephrine action for a few minutes

after each dose. More often, the maximum tolerated dosage was indicated by simultaneous appearance of signs of both epinephrine excess and CCK-179 overdosage. Ten patients were given full doses of Epinergone and, in a few, maintenance treatment was continued for over a month.

Figure 1 summarizes observations on 3 of the patients, which suggest that full doses of Epinergone sometimes were capable of substantially increasing adrenal cortical function. Mr. K. S. tolerated 16 to 24 mg./day of *l*-epinephrine and showed significant improvement in both psoriasis and rheumatoid arthritis with an accompanying definite increase in urinary 17-ketosteroid output; 40 units per day of long-acting ACTH failed to produce comparable clinical or laboratory changes. In Mrs. C. G., similar *l*-epinephrine dosage led to marked eosinopenia and hemodilution, together with rapid clearing of an acute optic neuritis. The induction of remission in previous similar instances of acute optic neuritis had required from 100 to 320 units/day of long-acting ACTH for at least one ten-day period (4). Mr. A.H., at the time he received Epinergone, had already been in the hospital for some weeks because a severe exacerbation of a life-long atopic eczema had failed to respond to treatment. He also presented intrinsic asthma and a marked allergic eosinophilia. In association with treatment, there was immediate subjective relief of itching, and objective improvement in his eczema was sufficiently rapid to permit his discharge as an ambulatory patient on the eighth treatment day. During treatment, the circulating eosinophil level fell from over 4,500 to less than 1,500 per cu.mm., and vital capacity showed a considerable increase.

Equally dramatic results were not obtained in all patients who received full doses of Epinergone. Three rheumatoid arthritis patients failed to show significant clinical or laboratory evidence of increased adrenal function. In the remaining 4 patients, laboratory changes were equivocal or therapeutic results were distinctly inferior to those obtainable with nominal dosage of cortisone or corticotropin.

The irregularity of results, relatively high cost, and inconvenience of giving four doses each day, make Epinergone unpromising for treatment. However, the clinical and laboratory changes observed during its use do suggest that sufficiently large doses of *l*-epinephrine can often induce an appreciable increase in human ACTH secretion. At best the increases are irregular and, are seen only with doses of *l*-epinephrine which are presumably unphysiologically large, since their administration is made possible only by giving a pharmacologic antagonist at the same time. Epinergone in full doses does not seem comparable to surgery or other systemic stresses as an activator of the pituitary-adrenal system. The findings are consistent with the view that *l*-epinephrine, in man, is not

important as a factor in the prompt release of large amounts of ACTH after sudden acute stress, or in the sustained increase in pituitary-adrenal activity induced by prolonged systemic stress.

WILLIAM Q. WOLFSON M.D.

Rackham Arthritis Research Unit,
University Hospital,
Ann Arbor, Michigan

Acknowledgment

Generous supplies of CCK-179 and other ergot derivatives used in these studies were made available through the cooperation of Dr. E. Henze and Mr. K. Ericson of Sandoz Pharmaceuticals.

REFERENCES

1. MEYER, RICHARD J.: Relative insensitivity of the hypothalamic-pituitary-adrenal system to activation by epinephrine, (Letter to the Editor) *J. Clin. Endocrinol. & Metab.* **13:** 123 (Jan.) 1953.
2. TEPPERMAN, J., and TEPPERMAN, H. M.: Metabolic functions of the endocrine glands, *Ann. Rev. Physiol.* **12:** 503–536, 1950.
3. WOLFSON, W. Q.: Unpublished observations.
4. QUINN, J. R., and WOLFSON, W. Q.: Improved results from individualized intensive hormonal treatment in certain eye diseases reported to respond poorly to ACTH or cortisone, *Univ. Michigan Med. Bull.* **18:** 1–21 (Jan.) 1952.

HYPOANDROGENIC SYNDROME WITH NORMAL SPERMATOGENESIS

TO THE EDITOR:

Among the papers presented at the Thirty-Fourth Annual Meeting of the Endocrine Society, Drs. E. P. McCullagh and J. C. Beck (1) reported 4 cases of a syndrome of eunuchoidism with spermatogenesis and normal FSH.

McCullagh and Beck state that although the possible existence of such cases has been hinted at repeatedly, to their knowledge none has been described. I, therefore, take the liberty of pointing out that in October of 1949, we presented a similar case to the Argentine Society of Endocrinology, the report of which has been published (2). Our patient was a man, 24 years of age, with a well defined hypoandrogenic syndrome—eunuchoid somatic proportions (height, 172 cm.; vertex-pubis, 72 cm.; pubis-sole, 100 cm.; span, 191 cm.) and lipomastia without gynecomastia. He had neither beard nor body hair; pubic hair was scarce and axillary hair very scarce. The penis was smaller than normal; the testes were of normal dimensions; there were erections but no spermogram could be carried out because of a psychological aversion to the procedure.

A testicular biopsy demonstrated that in 82 per cent of the seminiferous tubules there was active spermatogenesis, with spermatozoa; in 5 per cent, only a Sertoli syncytium was present; and in the remaining tubules there was incomplete spermatogenesis. The Sertoli cells showed nuclear vacuolization and mottled cytoplasm containing lipids but no birefringent steroids. The basal membrane of a number of tubules showed thickening to two or three layers of reticulin fibrils. The Leydig cells were scarce; the cytoplasm was compact and intensely acidophilic; it showed a positive reaction to Sudan III but there were no birefringent steroids.

Urinary FSH determinations gave results of >6, >12, <24, <48 mouse units. Two determinations of 17-ketosteroids at a three-month interval gave values of 4.6 and 11.6 mg. per twenty-four hours. The patient was then treated with 1,000 units of chorionic gonadotropin daily for twenty-one days. This brought about a marked increase in the excretion of 17-ketosteroids—13.8 mg. on the sixth day of treatment, 16.1 mg. on the twelfth day, and 21.9 mg. on the twenty-first day; ten days later the values were down to 14.1 mg. At the same time the patient seemed to improve psychologically, showing signs of greater decisiveness and impulsiveness. No signs of somatic changes were evident.

Our pathogenic interpretation of this case was at the time (2) similar to that now proposed by McCullagh and Beck, that is, a decrease in ICSH (LH) with a probably normal elaboration of FSH, the replacement therapy with chorionic gonadotropin causing an increase in the excretion of 17-ketosteroids. The thickening of the tubular wall appears to follow the decreased elaboration of testosterone. We believe that more cases of this syndrome will come to light if testicular biopsies are carried out on macroscopically normal testes when there is evidence of androgenic insufficiency.

RODOLFO Q. PASQUALINI, M.D.

Instituto Regional de Endocrinología,
Godoy Cruz 1221,
Buenos Aires, Argentina

REFERENCES

1. MCCULLAGH, E. P., and BECK, J. C.: A syndrome of eunuchoidism with spermatogenesis and normal urinary FSH, (abstract) *J. Clin. Endocrinol. & Metab.* **12**: 947, 1952.
2. PASQUALINI, R. Q., and BUR, G. E.: Sindrome hipoandrogénico con gametogénesis conservada, *Rev. Asoc. méd. argent.* **64**: 6, 1950.

Questions and Answers

(Answers to questions turned in at the 1952 Postgraduate Assembly of *The Endocrine Society*.)

QUESTION: What can be done for a patient with breast cancer who refuses irradiation and surgical treatment?

ANSWER: It is our feeling that the hormone treatment of breast cancer should be reserved only for incurable cases and the treatment should be symptomatic. Either androgens or estrogens will occasionally activate breast cancer so that it will grow faster and such therapy may be dangerous in that respect. The patient should be sterilized if she is premenopausal; this would also entail either radiation or surgical management. If the patient is premenopausal or approaching the menopause, testosterone may be of some benefit—again, with the realization that it occasionally enhances tumor growth. If the patient is in the very elderly age group (over 70-80), stilbestrol in a dose of 5 milligrams a day may be tried; definite regression will occur in about 50 per cent of the patients. Again, even in this age group, there is some danger of activation of the tumor with this drug; it would be preferable to leave the patient alone without the treatment until symptomatic problems demand palliation.

D. P. SLAUGHTER

QUESTION: At what age would you start A.P.L. therapy for cryptorchidism?

ANSWER: The glandular treatment of cryptorchidism should be started at the earliest age at which it is practical to carry out operative procedures, in case the glandular therapy is not effective in producing descent. In general, the age of 3 years seems to be the optimum one, although it should be pointed out that the earlier the testis can be brought into the scrotum, the greater its functional capacity is likely to be. The testis cannot function normally except in the environment of the scrotum and the earlier it is brought into its normal environment, the better. About 25 per cent of true undescended testes descend with glandular therapy. In the remainder, operative procedures have to be carried out; but preoperative glandular therapy makes it much easier to get the testis into the scrotum at the time of operation.

W. O. THOMPSON

QUESTION: How do you treat unilateral cryptorchidism, 1) prepuberal, 2) postpuberal?

ANSWER: The treatment of unilateral cryptorchidism before the age of puberty is the same as the treatment of bilateral cryptorchidism, except that the therapeutic agent may have to be different in patients with bilateral intra-abdominal testes. Chorionic gonadotropin is the material of choice, but in patients in whom both testes are in the abdominal cavity, this material may be ineffective. Under these circumstances, androgen therapy should be employed. After the age of puberty, glandular therapy is not necessary in unilateral cryptorchidism, except to correct hypogonadism if it is present. If the development of secondary sexual characteristics is normal, operative procedures may be carried out without first giving glandular treatment.

W. O. THOMPSON

QUESTION: Please discuss the medical treatment in advanced cases of testicular tumor.

ANSWER: It is assumed from the question that extensive metastases are already present. If such is the case it is very improbable that much can be done to extend the life or comfort of the patient. Although seminomas are more radiosensitive than teratomas, Boden and Giff of England (*Lancet*, **2**: 1195–1197 (Dec. 29) 1951) state that every case of testicular neoplasm which has been treated by orchiectomy should have x-ray therapy, irrespective of the nature of the tumor or the presence of metastases. Recently DeT. Shivers and Axelrog (*J. Urol.* **67**: 537–542 (Apr.) 1952) reported a case of seminoma which showed improvement in metastasis after the administration of estrogens and x-ray therapy. It is questionable, however, whether the life of the patient has been much extended. Since the majority of these patients fail very rapidly, adequate doses of sedatives should be used to relieve their pain.

N. J. HECKEL

QUESTION: Please discuss biopsy of the testis by puncture.

ANSWER: There is general agreement that the so-called open method is far superior to, and probably less troublesome to the patient, than the puncture method. One of its advantages is that a better specimen can be obtained for study. The technique is simple; a small incision is made through the skin, tunica vaginalis and tunica albuginea of the testis; a small wedge-shaped piece is removed with a pair of manicure scissors and immediately placed in Bouin's solution. The various layers just mentioned are closed with 0000 atraumatic catgut. Local or general anesthesia is used.

N. J. HECKEL

QUESTION: How long can you give large doses of gonadotropin before you destroy the germinal epithelium?

ANSWER: Much has been written on the effect of the administration of gonadotropin on the germinal epithelium of birds and animals. One of the most recent articles is by Pfeiffer (*Yale J. Biol. & Med.* **24**: 401 (Apr.) 1952). Little information has been published, however, regarding the effect of this hormone on the mature human testis. The most recent report has been that of Maddock and Nelson (*J. Clin. Endocrinol. & Metab.* **12**: 985–1007 (Aug.) 1952). They found that the administration of 5,000 units of chorionic gonadotropin three times a week for as short a period as forty-eight days would produce marked damage to the seminiferous tubules.

N. J. HECKEL

QUESTION: There are still a few localities where it is not possible to get I^{131} studies or 17-ketosteroid determinations on a domestic basis. Is there any work in progress concerning a screening test for 17-ketosteroid determination, *i.e.*, a test which would show low, normal or excessive amounts, and which could be carried out in smaller communities?

ANSWER: Such a screening test is not available as far as is known. Unless some new principle is discovered, it seems doubtful that a rough test would be sufficiently reliable for any purpose. In the use of any of the present methods for determination of the 17-ketosteroids, meticulous care in the measurement of solutions and in the purification of reagents is essential for accurate determinations. Results obtained with an inaccurate method may be greatly misleading, even though it is desired to detect only gross changes from the normal range of values.

H. L. MASON

The Endocrine Society

THE 1953 ANNUAL MEETING

The Thirty-fifth Annual Meeting of The Endocrine Society will be held in the Hotel Statler, New York, N. Y., on Thursday, Friday, and Saturday, May 28, 29 and 30, 1953.

The Committee on Local Arrangements is Dr. Earl T. Engle, Chairman, with Drs. Rulon W. Rawson and Sidney C. Werner as members of the Committee.

All Scientific Sessions will be held in the Hotel Statler. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Reservations should be made directly with the Hotel Statler, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send FOUR COPIES of the title and abstract to the Vice-President, Dr. Lawson Wilkins, Johns Hopkins Hospital, Baltimore 5, Maryland, not later than FEBRUARY 1, 1953. **It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:**

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three additional copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

The Endocrine Society

1953 AWARDS AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1953 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1954.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson in 1950. Dr. John D. Stoekle was chosen as the recipient of the 1951 Fellowship. Inasmuch as he was unable to accept, due to a call to military service, the same Fellowship was reawarded in 1952 to Dr. C. L. Courtright. The 1953 Schering Fellowship will be in the amount of $5,000 and will henceforth be given in the same amount on alternate years.

Awards

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH AWARD

This Award of $1,000 was established in 1939 by The Squibb Institute for Medical Research and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. None was given in 1943. This Award was increased to the amount of $2,500 beginning with the 1952 Award and the recipient was Dr. James H. Means. A special committee of five members of the Society selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1953.*

The American Goiter Association

1953 ANNUAL MEETING

The 1953 Annual Meeting will be held *May 7, 8 and 9, 1953*, in the Drake Hotel, Chicago, Illinois.

VAN METER PRIZE AWARD FOR 1953

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association, which will be held in Chicago, Illinois, May 7, 8 and 9, 1953, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, not later than *February 15, 1953*. The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association.

BOOKS RECEIVED

American Lecture Series. Charles C Thomas, Publisher, Springfield, Illinois.

Diabetic Glomerulosclerosis. By HAROLD RIFKIN, M.D., Lecturer in Medicine, College of Physicians and Surgeons, Columbia University; Adjunct Attending Physician, Montefiore Hospital, New York, LOUIS LEITER, M.D., Ph.D., Clinical Professor of Medicine, College of Physicians and Surgeons, Columbia University; Chief, Medical Division, Montefiore Hospital, and JAMES BERKMAN, M.D., Instructor in Pathology, College of Physicians and Surgeons, Columbia University; Associate Pathologist, Montefiore Hospital. A monograph in American Lectures in Metabolism, edited by Paul György, M.D., Louis Leiter, M.D. and S. O. Waife, M.D.; 112 pages; 12 tables; 13 illustrations; 1952. Price $3.50.

Diagnosis and Treatment of Adrenal Insufficiency. Japanese edition. By GEORGE W. THORN, M.D., Hersey Professor of the Theory and Practice of Physic, Harvard Medical School, and Physician-in-Chief, Peter Bent Brigham Hospital, Boston, Mass., PETER H. FORSHAM, M.D., Instructor in Medicine, Harvard Medical School, and KENDALL EMERSON, JR., Asst. Professor, Harvard Medical School (both of the Peter Bent Brigham Hospital). A monograph in American Lectures in Endocrinology, edited by W. O. Thompson, M.D.; 180 pages, 1952. ¥ 450.

Infrared Photography in Medicine. By LEO C. MASSOPUST, SR., Director, Department of Art and Photography, Marquette University School of Medicine, Milwaukee, Wisconsin. A monograph in American Lectures in Medical Photography, edited by Ralph P. Creer; 62 pages; 31 illustrations; 1952.

Physiological Bases of Gynecology and Obstetrics. By S. R. M. REYNOLDS, Ph.D., D.Sc., Staff Member, Physiologist, Department of Embryology, Carnegie Institution of Washington; Lecturer in Obstetrics, The Johns Hopkins Medical School, Baltimore, Maryland. A monograph in American Lectures in Gynecology and Obstetrics, edited by E. C. Hamblen; 165 pages; 1952. Price $5.50.

Rare Manifestations of Metabolic Bone Disease. By I. SNAPPER, M.D., Ph.D., Physician and Director of Medical Education, Mt. Sinai Hospital; Clinical Professor of Medicine, Columbia University College of Physicians and Surgeons, New York. A monograph in American Lectures in Metabolism, edited by Paul György, M.D., Louis Leiter, M.D. and S. O. Waife, M.D.; 101 pages; 11 tables; 18 illustrations; 1952. Price $3.00.

Maladies des globules rouges et désordres endocriniens. Par A. ASCHKENASY, Chef de laboratoire à Hôtel-Dieu, Chargé de Recherches au Centre National de la Recherche Scientifique; 112 pages; 5 tableaux; 6 figures; 1952. G. Doin & Cie, Paris. 700 fr.

(*The*) *Mechanisms of Disease. A study of the Autonomic Nervous System, the Endocrine System and the Electrolytes in Their Relationship to Clinical Medicine.* By JOSEPH STAMBUL, M.D., Chief of the Cardiovascular Department of Albert Einstein Medical Center (Eastern Division), and Cardiologist to Deborah Sanatorium, with a foreword by GEORGE MORRIS PIERSOL, M.D., Professor of Medicine, Graduate School of Medicine, University of Pennsylvania; 780 pages; 1952. Froben Press, Inc., New York. Price $15.00.

Oestrogens and Neoplasia. By HAROLD BURROWS, C. B. E., Ph.D., F.R.C.S., The Chester Beatty Research Institute, the Royal Cancer Hospital, London, England, and ERIC HORNING, M.A., D.Sc., Reader in Experimental Pathology in the University of London, at the Royal Cancer Hospital, with a foreword by CHARLES HUGGINS, M.D., Professor of Surgery, University of Chicago; 204 pages; 42 tables; 1952. Charles C Thomas, Publisher, Springfield, Illinois. Price $6.75.

THREE NEW SECTIONS ADDED TO THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM

At the meeting of the Editorial Board of *The Journal of Clinical Endocrinology and Metabolism,* held at the Palmer House in Chicago in June, 1952, it was decided that three new sections should be added:

1. A section on teaching clinics in endocrinology.
2. An editorial section.
3. A question-and-answer section.

The teaching clinics are to be devoted to discussions of cases that are of interest from a teaching and diagnostic point of view. This section does not concern itself primarily with rare conditions. In fact, commonplace disorders may be discussed in this section because of their diagnostic and teaching value.

The editorial section represents an expansion of a section which has been incorporated in the Journal from time to time but not developed.

The question-and-answer section will present questions turned in at the Postgraduate Assembly of the Endocrine Society and answered by outstanding authorities in various subdivisions of endocrinology.

The section on teaching clinics will not be started until the February or March issue. The editorial and the question-and-answer sections begin with this issue.

These three sections are being added, in order to increase the interest of the average practicing physician in this Journal. It is the opinion of the Editorial Board that the publication of original scientific manuscripts is the most important function of the Journal, but the Board also feels strongly that the Journal should be a teaching medium for clinical endocrinology and metabolism, and that every effort should be made to increase its usefulness for the practitioner of medicine.

W. O. Thompson, M.D.
Managing Editor

STERILITY

Its Cause and Its Treatment

By J. JAY ROMMER, M.D.

Gynecological Staff, Beth Israel Hospital, Newark, New Jersey

This book sharply defines the mechanism of sterility and shows how often barrenness may be converted into fertility.

Part I
THE INFERTILE FEMALE

Part II
THE INFERTILE MALE

EVERY KNOWN cause of sterility is covered, plus many factors found in the author's own research on the subject; physical, mental, environmental, psychological, endocrine, gynecological, surgical, and congenital causes are explained with the method of investigation for each of them. *Complete laboratory, roentgenological, and other investigational procedures which will help in tracking down the cause are presented.* In this compact book is crystallized all existing thinking and knowledge on the CAUSE and TREATMENT OF STERILITY.

With this book there is no need to hunt through medical journals for hints on the treatment of sterility. Here is a *definitive treatise* which embraces *all aspects of the reproductive problem.* By pointing out significant contributions being made to this subject by various medical and allied specialties, together with many personal observations, the author brings into better perspective the subject of reproductive failures and control.

This presentation is a comprehensive coverage of an extremely complicated and intricate subject. It brings together in a single volume much highly useful information on fertility and sterility which is of value not only to various individuals with diverse interest (such as gynecologists, urologists, internists, endocrinologists, psychiatrists, pathologists, physiologists, dieticians, anatomists, and others), but also provides the practicing physician with many valuable clinical procedures for adaptation to the care of his patients.

427 pages *259 illustrations* *Price: $12.50, postpaid*

CHARLES C THOMAS · PUBLISHER · SPRINGFIELD · ILLINOIS